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(54) Title: ALPHAVIRUS VECTORS

(57) Abstract

A modified alphavirus expression vector is provided wherein at least one optimal heterologous splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus, which may be Semliki Forest virus following administration of the vector to a host.

TITLE OF INVENTION

ALPHAVIRUS VECTORS

FIELD OF INVENTION

The present invention relates to the field of DNA vaccines and is particularly concerned with modified alpha virus vectors for use in such vaccines.

BACKGROUND OF THE INVENTION

Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. The mature virus particle contains a single copy of a ssRNA genome with a positive polarity that is 5'-capped and 3'-polyadenylated. It functions as an mRNA and naked RNA can start an infection when introduced into cells. Upon infection/transfection, the 5' two-thirds of the genome is translated into a polyprotein that is processed into the four nonstructural proteins (nsP1 to 4) by self cleavage. Once the ns proteins have been synthesized they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 14). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S subgenomic mRNA (ref. 1 - Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification. The disclosures of these references are hereby incorporated by reference into the present disclosure). This subgenomic mRNA, which is colinear with the last one-third of the genome, encodes the SFV structural

proteins. In 1991 Liljestrom and Garoff (ref. 2) designed a series of expression vectors based on the SFV CDNA replicon. These vectors had the virus structural protein genes deleted to make the way for heterologous inserts, but preserved the nonstructural coding region for production of the nsPl to 4 replicase complex. Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by translation stop sites in all three frames. An SpeI site was inserted just after the 3' end of the SFV CDNA for linearization of the plasmid for use *in vitro* transcription reactions.

Injection of SFV RNA encoding a heterologous protein have been shown to result in the expression of the foreign protein and the induction of antibody in a number of studies (refs. 3,4). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages associated with plasmid DNA immunization. For example, SFV RNA encoding a viral antigen may be introduced in the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the virus. Also, because the protein is expressed *in vivo* the protein should have the same conformation as the protein expressed by the virus itself. Therefore, concerns about conformational changes which could occur during protein purification leading to a loss in immunogenicity, protective epitopes and possibly immunopotentiation, could be avoided by plasmid DNA immunization.

In WO95/27044, the disclosure of which is incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once transcribed from the cDNA under transceptional control of a heterologous promoter, the alphavirus RNA is able to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

SUMMARY OF THE INVENTION

The present invention is concerned with modifications to the alphavirus cDNA vectors described in the aforementioned WO 95/27044 to permit enhanced replication of the alphavirus. In the present invention, a heterologous splice site is introduced into the alphavirus replicon sequence, particularly that of Semliki Forest virus (SFV).

Accordingly, in one aspect, the present invention provides an expression vector comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA, and further comprises a heterologous DNA sequence capable of expression in a suitable host, such as a human or animal host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said animal or human host, wherein at least one heterologous splice site is

provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

The alphavirus molecule is a large molecule and, accordingly, there is a high probability of cryptic splice sites, thereby impairing the replication of the alphavirus and hence its ability to express the heterlogous DNA is impaired. By introducing the at least one optimal heterologous splice site in accordance with the present invention into the alphavirus replicon sequence, any splicing is likely to be directed at the heterologous splice site rather than any cryptic splice sites, restores the function of the SFV replicon when removed, and may improve transport of RNA from the nucleus (ref. 6).

In the constructs provided herein, the promoter is placed upstream of the 5'-end of the alphavirus sequence, such that the resultant transcript has an authentic 5'-end, which is required for the efficient replication of the alphavirus RNA replicon.

In addition, there may be provided at the 3'end of the Semliki Forest virus segment, a hepatitis delta virus ribozyme sequence to ensure proper *in vivo* cleavage at the 3'-end of the sequence. Any other convenient sequence may be employed to achieve this effect.

The heterologous splice site sequence may be provided by the nucleotide sequence of the rabbit β-globin intron II, as described in reference 5. Such heterologous splice site sequence may be inserted into the complement sequence at any convenient location which generates perfect splice junctions. This

precludes replication of the alphavirus, unless it is authentically removed by splicing..

I have identified five suitable sites in the SFV replicon, which are contained within an EcoRV-SpeI fragment of the replicon which is 8010 bp in length (Fig. 3). The first such site is a Ppu-MI site, at position 2719 within the EcoRV-SpeI fragment.

In constructing the modified vectors provided herein, the EcoRV-SpeI fragment is cut with Ppu-MI at position 2719 and made blunt-ended with Mung Bean nuclease, which removes three bases from the SFV sequence. A blunt-ended β-globin II intron, which is 536 bp long, is ligated into the site and replaces the missing three bases with sequence added to the 3'-end of the β-globin intron sequence (Fig. 1).

The other four suitable sites for insertion of the Intron are the PvuII sites at bp 2518, 3113, 6498 and 6872 of the EcoRV-SpeI fragment. Insertion of the Intron is achieved by cutting with PvuII (a blunt end cutter) and the blunt-ended β-globin II intron sequence (Fig. 2) is ligated into one or more of these sites.

In a further aspect of the present invention, there is provided a cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is

non-essential to replication thereof; a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript had an authentic 5' end; at least one heterologous splice set provided in the complement of the DNA molecule to generate perfect splice junctions in the alphavirus in order to prevent aberrant splicing and an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant mRNA transcript.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the DNA sequence of the β-globin intron II including three additional nucleotides at the 3'-end thereof (SEQ ID No:1);

Figure 2 shows the DNA sequence of the β-globin intron II (SEQ ID No:2);

Figures 3A to 3C show the DNA sequence of the EcoRV-SpeI fragment of Semliki Forest virus replicon (SEQ ID No:3);

Figures 4A to 4D show the DNA sequence of the pSFV link (SEQ ID no: 4) prepared as illustrated in Figure 5;

Figure 5 shows construction of pSFVlink (11060 bp) from pSFV1 using a linker sequence (SEQ ID nos: 5,6);

Figures 6A to 6D show the nucleotide sequence of plasmid pMP76 (SEQ ID no: 11, prepared as illustrated in Figures 8A to 8D;

Figure 7 illustrates subsections of plasmid pSFV link (see Figure 5);

Figure 8A to 8D show the construction of plasmid pMP76 from plasmids pMP53, pMP70, pMP47, pMP55 and pMP71;

Figures 9A to 9B show the construction of plasmids pMP53, pMP54 and pMP55 from plasmid pMP52;

Figure 10 shows the construction of plasmid MP52 from pUC19 using a linker sequence (SEQ ID no: 7,8);

Figures 11A to 11B show the construction of plasmids pMP46, pMP47 and pMP70 from pUC19 and fragment from pSFV link, prepared as seen in Figure 7; and

Figures 12A to 12B show the construction of plasmid pMP71 from plasmid pCMV3.

GENERAL DESCRIPTION OF INVENTION

As discussed above, the present invention provides a modified alphavirus DNA. The alphavirus preferably is Semliki Forest virus. In particular, the present invention provides a cloning vector for heterologous gene expression in a host, such as an animal or human.

The promoter sequence may comprise a promoter of eukaryotic or prokaryotic origin. Suitable promoters are the cytomegalovirus immediate early promoter (pCMV), although other promoters, such as the Rous sarcoma virus long-terminal repeat promoter (pRSV), since, in the case of these and similar promoters, transcription is performed by the DNA-dependent RNA polymerase of the host cell. Additionally, the SP6, T3 or T7 promoters can be used, provided that the cell has first been transformed with genes encoding SP6, T3 or T7 RNA polymerase molecules which are either inserted into the chromosome or remain episomal. Expression of

these (SP6, T3, T7) RNA polymerase-encoding genes is dependent on the host cell DNA-dependent RNA polymerase.

The heterologous DNA insert may comprise the coding sequence for a desired product, which may be a biologically active protein or polypeptide, for example, the heterologous DNA insert may code for HIV sequences, e.g., an immunogenic or antigenic protein or polypeptide, or a therapeutically active protein or polypeptide. The heterologous DNA may also comprise additional sequences, such as a sequence complementary to an RNA sequence which is a self-cleaving ribozyme sequence.

The DNA vectors provided herein may be administered to a host, including a human host, for *in vivo* expression of the heterologous DNA sequence, in accordance with a further aspect of the invention, in order to generate an immune response in the host, which may be a protective immune response. The DNA vectors may be further formulated into immunogenic compositions for such administration.

BIOLOGICAL DEPOSITS

Certain vectors that contain the Semliki Forest virus replicon and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

Samples of the deposited plasmids will become available to the public upon grant of a patent based

upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Non-viable deposits will be replaced. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention.

Deposit Summary

Plasmid	ATCC Designation	Date Deposited
pMP76		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

EXAMPLE 1

This Example describes the construction of plasmid pMP76 as outlined in Figures 5, 7, 8A, 8B, 8C, 8D, 9A, 9B, 10, 11A, 11B, 12A and 12B.

Plasmid pSFV link was created by restricting plasmid pSFV1 (Gibco) with BamHI. This plasmid was then ligated with a linker (SEQ ID no: 5 and 6) to produce plasmid pSFV link (Figures 4A to 4D, Figure 5).

Some of the SFV replicon fragments were subcloned by restricting pSFVlink with EcoRV and SpeI and isolating the 890bp EcoRV-SpeI fragment. This fragment was then restricted with EcoRI and the 1906bp EcoRV-EcoRI, the 1578bp and 3627bp EcoRI-EcoRI and the 899bp EcoRI-SpeI fragments isolated (Fig.7).

The 1909bp EcoRV-EcoRI SFV fragment was cloned into EcoRV-EcoRI restricted plasmid pMP52 to produce plasmid pMP53 (Fig.9A). The 899bp EcoRI-SpeI SFV fragment was cloned into EcoRI-SpeI restricted pMP52 to produce pMP54 (Fig.9A). Plasmid pMP54 was then restricted with SpeI and made blunt-ended with Mung Bean nuclease. The plasmid was then restricted with BglII, dephosphorylated and ligated to the hepatitis delta virus ribozyme linker (SEQ ID nos. 9 and 10), that had been phosphorylated, to produce pMP55 (Fig. 9B).

Plasmid pMP52 was created by ligating a linker (SEQ ID nos:7,8), into the EcoRI site of pUC19 (Fig.10).

The 1578bp EcoRI-SFV fragment ws cloned into the EcoRI site of pUC19, to produce pMP46 (Fig.11A). This plasmid was then restricted with PpuM1 and made

blunt-ended with Mung Bean nuclease. The rabbit ß-globin intron II PCR fragment (Fig.1) was made blunt-ended with Mung Bean nuclease, phosphorylated and ligated to the PpuMI restricted pMP46 to produce plasmid pMP70 (Fig.11B).

The 3627bp EcoRI SFV fragment was cloned into the EcoRI site of pUC19 to produce pMP47 (Fig.11A).

Plasmid pCMV3, which contains the CMV promoter, Intron A sequence, BGH poly A sequence and SU40 poly A sequence, was restricted with NdeI and EcoRV. The 3191bp NdeI-EcoRV fragment was isolated and dephosphorylated. The 1321bp NdeI-EcoRV fragment was isolated and restricted with SacI. The NdeI-SacI fragment of 334bp was isolated (Fig.12A). The isolated SacI-EcoRV PCR fragment containing the 5'-end of SFV was ligated to the previously isolated 334bp NdeI-SacI fragment and the 3191bp NdeI-EcoRV fragment to produce pMP71 (Fig.12A and 12B).

Plasmid pMP53 was then restricted with EcoRI and BamHI and ligated to the isolated and dephosphorylated 2151bp EcoRI fragment from pMP70 (Fig.8A). This ligation was then restricted with EcoRV and the 4057bp EcoRV-EcoRI fragment purified(Fig.8A).

Plasmid pMP47 was restricted with EcoRI and the 3627bp EcoRI fragment isolated and dephosphorylated (Fig.8B). Plasmid pMP55 was then restricted with BglII, dephosphorylated and restricted with EcoRI. The 985bp EcoRI-BglII fragment was isolated and ligated to the previously isolated EcoRI fragment from pMP47 (Fig.8B). The ligation reaction was then

phosphorylated and the 4612bp EcoRI-BglII fragment isolated.

Plasmid pMP71 was restricted with EcoRV and BamHI then dephosphorylated. This fragment was used in a 3-way ligation with the previously isolated 4612bp EcoRI-BglII fragment from pMP47 and pMP55, and the 4057bp EcoRV-EcoRI fragment from pMP53 and pMP70, to produce pMP76 (Figs.8B and 8C).

The 5' end of the SFV replicon was produced by PCR amplification of pSFV1 using primers SFV-5'-3 having the sequence 5'-ATCTATGAGCTCGTTTAGTGAACCGTATGGCGGATGTGTGACATACA-3' and EcoR-SPE having the sequence 5'-TCCACCTCCAAGGATATCCAAGATGAGTGTG-3' (SEQ ID no: 9 and SEQ ID no: 10 respectively) between the CMV promoter and the 5' end of the SFV replicon. The resulting PCR fragment was restricted with SacI and EcoRV (Fig. 13; SEQ ID no: 11) and the fragment isolated.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a modified alphavirus-based expression vector wherein at least one optimal splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus genome; and improve transport of RNA out of the nucleus. Modifications are possible within the scope of the invention.

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CLAIMS

1. An expression vector, comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA and further comprises a heterologous DNA seuence capable of expression in a host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said host, wherein at least one heterologous splice site is provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

2. The vector of claim 1 wherein said promoter is placed upstream of the 5'-end of the DNA molecule such that the resultant transcript has an authentic 5'-end.

3. The vector of claim 2 wherein said promoter is the cytomegalovirus immediate early promoter.

4. The vector of claim 1 which further comprises an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the DNA molecule.

5. The vector of claim 4 wherein said additional DNA sequence comprises a hepatitis delta ribozyme sequence.

6. The vector of claim 1 wherein the heterologous splice site sequence is provided by the DNA sequence of the rabbit ß-globin intron II.

7. The vector of claim 6 wherein the heterologous splice site sequence is inserted into the DNA molecule

at a location which generates perfect splice junctions and restores the function of the SFV replicon when removed.

8. The vector of claim 1 wherein the alphavirus is a Simliki Forest virus.

9. A cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises:

a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is non-essential to replication thereof;

a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript had an authentic 5' end;

at least one heterologous splice set provided in the complement of the DNA molecule to permit aberrant RNA splicing of one to generate perfect splice junctions in the alphavirus; and

an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant RNA molecule.

10. The cloning vector of claim 9 wherein said heterologous splice set is provided by the DNA sequence of the rabbit ß-globin intron II.

11. The cloning vector of claim 9 wherein said additional sequence comprises a hepatitis delta ribozyme sequence.

12. The cloning vector of claim 8 wherein the alphavirus is a Semliki Forest virus.

13. The cloning vector of claim 8 which has the identifying characteristics of plasmid pMP76 shown in Figure 8D.

14. The cloning vector of claim 8 having SEQ ID no: 11.

FIG.1

Nucleotide Sequence of the β-globin intron II with the 3' SFV bases

```
gtgagtttgg ggacccttga ttgttctttc tttttcgcta ttgtaaaatt catgttatat 60
ggagggggca aagttttcag ggtgttgttt agaatgggaa gatgtccctt gtatcaccat 120
ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct 180
cttattttct tttcattttc tgtaactttt tcgttaaact ttagcttgca tttgtaacga 240
atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300
tttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaattgtt 360
ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt 420
cttattggta gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540
aaccatgttc atgccttctt ctttttccta caggtc                           576
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FIG.2

Nucleotide Sequence of the β-globin intron II

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gtgagtttgg ggacccttga ttgttctttc tttttcgcta ttgtaaaatt catgttatat 60
ggagggggca aagttttcag ggtgttgttt agaatgggaa gatgtccctt gtatcaccat 120
ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct 180
cttattttct tttcattttc tgtaactttt tcgttaaact ttagcttgca tttgtaacga 240
atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300
tttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaattgtt 360
ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt 420
cttattggta gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540
aaccatgttc atgccttctt ctttttccta cag                               573
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FIG.3A

Eco RV-SpeI Fragment of Semliki Forest virus replicon

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atcggcagtg cgccttccag gagaatgatg tctacgcaca aataccactg cgtatgccct 60
atgcgcagcg cagaagaccc cgaaaggctc gatagctacg caaagaaact ggcagcggcc 120
tccgggaagg tgctggatag agagatcgca ggaaaaatca ccgacctgca gaccgtcatg 180
gctacgccag acgctgaatc tcctaccttt tgcctgcata cagacgtcac gtgtcgtacg 240
gcagccgaag tggccgtata ccaggacgtg tatgctgtac atgcaccaac atcgctgtac 300
catcaggcga tgaaaggtgt cagaacggcg tattggattg ggtttgacac caccccgttt 360
atgtttgacg cgctagcagg cgcgtatcca acctacgcca caaactgggc cgacgagcag 420
gtgttacagg ccaggaacat aggactgtgt gcagcatcct tgactgaggg aagactcggc 480
aaactgtcca ttctccgcaa gaagcaattg aaaccttgcg acacagtcat gttctcggta 540
ggatctacat tgtacactga gagcagaaag ctactgagga gctggcactt accctccgta 600
ttccacctga aaggtaaaca atcctttacc tgtaggtgcg ataccatcgt atcatgtgaa 660
gggtacgtag ttaagaaaat cactatgtgc cccggcctgt acggtaaaac ggtagggtac 720
gccgtgacgt atcacgcgga gggattccta gtgtgcaaga ccacagacac tgtcaaagga 780
gaaagagtct cattccctgt atgcacctac gtcccctcaa ccatctgtga tcaaatgact 840
ggcatactag cgaccgacgt cacaccggag gacgcacaga agttgttagt gggattgaat 900
cagaggatag ttgtgaacgg aagaacacag cgaaacacta acacgatgaa gaactatctg 960
cttccgattg tggccgtcgc atttagcaag tgggcgaggg aatacaaggc agaccttgat 1020
gatgaaaaac ctctgggtgt ccgagagagg tcacttactt gctgctgctt gtgggcattt 1080
aaaacgagga agatgcacac catgtacaag aaaccagaca cccagacaat agtgaaggtg 1140
ccttcagagt ttaactcgtt cgtcatcccg agcctatggt ctacaggcct cgcaatccca 1200
gtcagatcac gcattaagat gcttttggcc aagaagacca agcgagagtt aatacctgtt 1260
ctcgacgcgt cgtcagccag ggatgctgaa caagaggaga aggagaggtt ggaggccgag 1320
ctgactagag aagccttacc acccctcgtc cccatcgcgc cggcggagac gggagtcgtc 1380
gacgtcgacg ttgaagaact agagtatcac gcaggtgcag gggtcgtgga aacacctcgc 1440
agcgcgttga aagtcaccgc acagccgaac gacgtactac taggaaatta cgtagttctg 1500
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FIG.3B

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tccccgcaga ccgtgctcaa gagctccaag ttggcccccg tgcaccctct agcagagcag 1560
gtgaaaataa taacacataa cgggagggcc ggcggttacc aggtcgacgg atatgacggc 1620
agggtcctac taccatgtgg atcggccatt ccggtccctg agtttcaagc tttgagcgag 1680
agcgccacta tggtgtacaa cgaaagggag ttcgtcaaca ggaaactata ccatattgcc 1740
gttcacggac cgtcgctgaa caccgacgag gagaactacg agaaagtcag agctgaaaga 1800
actgacgccg agtacgtgtt cgacgtagat aaaaaatgct gcgtcaagag agaggaagcg 1860
tcgggtttgg tgttggtggg agagctaacc aaccccccgt tccatgaatt cgcctacgaa 1920
gggctgaaga tcaggccgtc ggcaccatat aagactacag tagtaggagt ctttggggtt 1980
ccgggatcag gcaagtctgc tattattaag agcctcgtga ccaaacacga tctggtcacc 2040
agcggcaaga aggagaactg ccaggaaata gttaacgacg tgaagaagca ccgcgggaag 2100
gggacaagta gggaaaacag tgactccatc ctgctaaacg ggtgtcgtcg tgccgtggac 2160
atcctatatg tggacgaggc tttcgcttgc cattccggta ctctgctggc cctaattgct 2220
cttgttaaac ctcggagcaa agtggtgtta tgcggagacc ccaagcaatg cggattcttc 2280
aatatgatgc agcttaaggt gaacttcaac cacaacatct gcactgaagt atgtcataaa 2340
agtatatcca gacgttgcac gcgtccagtc acggccatcg tgtctacgtt gcactacgga 2400
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cagttggact accgtggaca cgaagtcatg acagcagcag catctcaggg cctcacccgc 2580
aaaggggtat acgccgtaag gcagaaggtg aatgaaaatc ccttgtatgc ccctgcgtcg 2640
gagcacgtga atgtactgct gacgcgcact gaggataggc tggtgtggaa aacgctggcc 2700
ggcgatccct ggattaaggt cctatcaaac attccacagg gtaactttac ggccacattg 2760
gaagaatggc aagaagaaca cgacaaaata atgaaggtga ttgaaggacc ggctgcgcct 2820
gtggacgcgt tccagaacaa agcgaacgtg tgttgggcga aaagcctggt gcctgtcctg 2880
gacactgccg gaatcagatt gacagcagag gagtggagca ccataattac agcatttaag 2940
gaggacagag cttactctcc agtggtggcc ttgaatgaaa tttgcaccaa gtactatgga 3000
gttgacctgg acagtggcct gttttctgcc ccgaaggtgt ccctgtatta cgagaacaac 3060
```

FIG.3C

```
cactgggata acagacctgg tggaaggatg tatggattca atgccgcaac agctgccagg 3120
ctggaagcta gacatacctt cctgaagggg cagtggcata cgggcaagca ggcagttatc 3180
gcagaaagaa aaatccaacc gctttctgtg ctggacaatg taattcctat caaccgcagg 3240
ctgccgcacg ccctggtggc tgagtacaag acggttaaag gcagtagggt tgagtggctg 3300
gtcaataaag taagagggta ccacgtcctg ctggtgagtg agtacaacct ggctttgcct 3360
cgacgcaggg tcacttggtt gtcaccgctg aatgtcacag gcgccgatag gtgctacgac 3420
ctaagtttag gactgccggc tgacgccggc aggttcgact tggtctttgt gaacattcac 3480
acggaattca gaatccacca ctaccagcag tgtgtcgacc acgccatgaa gctgcagatg 3540
cttgggggag atgcgctacg actgctaaaa cccggcggca tcttgatgag agcttacgga 3600
tacgccgata aaatcagcga agccgttgtt tcctccttaa gcagaaagtt ctcgtctgca 3660
agagtgttgc gcccggattg tgtcaccagc aatacagaag tgttcttgct gttctccaac 3720
tttgacaacg gaaagagacc ctctacgcta caccagatga ataccaagct gagtgccgtg 3780
tatgccggag aagccatgca cacggccggg tgtgcaccat cctacagagt taagagagca 3840
gacatagcca cgtgcacaga agcggctgtg gttaacgcag ctaacgcccg tggaactgta 3900
ggggatggcg tatgcagggc cgtggcgaag aaatggccgt cagcctttaa gggagcagca 3960
acaccagtgg gcacaattaa aacagtcatg tgcggctcgt accccgtcat ccacgctgta 4020
gcgcctaatt tctctgccac gactgaagcg gaaggggacc gcgaattggc cgctgtctac 4080
cgggcagtgg ccgccgaagt aaacagactg tcactgagca gcgtagccat cccgctgctg 4140
tccacaggag tgttcagcgg cggaagagat aggctgcagc aatccctcaa ccatctattc 4200
acagcaatgg acgccacgga cgctgacgtg accatctact gcagagacaa aagttgggag 4260
aagaaaatcc aggaagccat tgacatgagg acggctgtgg agttgctcaa tgatgacgtg 4320
gagctgacca cagacttggt gagagtgcac ccggacagca gcctggtggg tcgtaagggc 4380
tacagtacca ctgacgggtc gctgtactcg tactttgaag gtacgaaatt caaccaggct 4440
gctattgata tggcagagat actgacgttg tggcccagac tgcaagaggc aaacgaacag 4500
atatgcctat acgcgctggg cgaaacaatg gacaacatca gatccaaatg tccggtgaac 4560
gattccgatt catcaacacc tcccaggaca gtgccctgcc tgtgccgcta cgcaatgaca 4620
```

FIG.3D

```
gcagaacgga tcgcccgcct taggtcacac caagttaaaa gcatggtggt ttgctcatct 4680
tttcccctcc cgaaatacca tgtagatggg gtgcagaagg taaagtgcga gaaggttctc 4740
ctgttcgacc cgacggtacc ttcagtggtt agtccgcgga agtatgccgc atctacgacg 4800
gaccactcag atcggtcgtt acgagggttt gacttggact ggaccaccga ctcgtcttcc 4860
actgccagcg ataccatgtc gctacccagt ttgcagtcgt gtgacatcga ctcgatctac 4920
gagccaatgg ctcccatagt agtgacggct gacgtacacc ctgaacccgc aggcatcgcg 4980
gacctggcgg cagatgtgca ccctgaaccc gcagaccatg tggacctcga gaacccgatt 5040
cctccaccgc gcccgaagag agctgcatac cttgcctccc gcgcggcgga gcgaccggtg 5100
ccggcgccga gaaagccgac gcctgcccca aggactgcgt ttaggaacaa gctgcctttg 5160
acgttcggcg actttgacga gcacgaggtc gatgcgttgg cctccgggat tactttcgga 5220
gacttcgacg acgtcctgcg actaggccgc gcgggtgcat atattttctc ctcggacact 5280
ggcagcggac atttacaaca aaaatccgtt aggcagcaca atctccagtg cgcacaactg 5340
gatgcggtcc aggaggagaa aatgtacccg ccaaaattgg atactgagag ggagaagctg 5400
ttgctgctga aaatgcagat gcacccatcg gaggctaata agagtcgata ccagtctcgc 5460
aaagtggaga acatgaaagc cacggtggtg gacaggctca catcgggggc cagattgtac 5520
acgggagcgg acgtaggccg cataccaaca tacgcggttc ggtacccccg ccccgtgtac 5580
tcccctaccg tgatcgaaag attctcaagc cccgatgtag caatcgcagc gtgcaacgaa 5640
tacctatcca gaaattaccc aacagtggcg tcgtaccaga taacagatga atacgacgca 5700
tacttggaca tggttgacgg gtcggatagt tgcttggaca gagcgacatt ctgcccggcg 5760
aagctccggt gctacccgaa acatcatgcg taccaccagc cgactgtacg cagtgccgtc 5820
ccgtcaccct ttcagaacac actacagaac gtgctagcgg ccgccaccaa gagaaactgc 5880
aacgtcacgc aaatgcgaga actacccacc atggactcgg cagtgttcaa cgtggagtgc 5940
ttcaagcgct atgcctgctc cggagaatat tgggaagaat atgctaaaca acctatccgg 6000
ataaccactg agaacatcac tacctatgtg accaaattga aaggcccgaa agctgctgcc 6060
ttgttcgcta agacccacaa cttggttccg ctgcaggagg ttcccatgga cagattcacg 6120
gtcgacatga aacgagatgt caaagtcact ccagggacga aacacacaga ggaaagaccc 6180
```

FIG.3E

```
aaagtccagg taattcaagc agcggagcca ttggcgaccg cttacctgtg cggcatccac 6240
agggaattag taaggagact aaatgctgtg ttacgcccta acgtgcacac attgtttgat 6300
atgtcggccg aagactttga cgcgatcatc gcctctcact tccacccagg agacccggtt 6360
ctagagacgg acattgcatc attcgacaaa agccaggacg actccttggc tcttacaggt 6420
ttaatgatcc tcgaagatct aggggtggat cagtacctgc tggacttgat cgaggcagcc 6480
tttggggaaa tatccagctg tcacctacca actggcacgc gcttcaagtt cggagctatg 6540
atgaaatcgg gcatgtttct gactttgttt attaacactg ttttgaacat caccatagca 6600
agcagggtac tggagcagag actcactgac tccgcctgtg cggccttcat cggcgacgac 6660
aacatcgttc acggagtgat ctccgacaag ctgatggcgg agaggtgcgc gtcgtgggtc 6720
aacatggagg tgaagatcat tgacgctgtc atgggcgaaa aacccccata tttttgtggg 6780
ggattcatag tttttgacag cgtcacacag accgcctgcc gtgtttcaga cccacttaag 6840
cgcctgttca agttgggtaa gccgctaaca gctgaagaca agcaggacga agacaggcga 6900
cgagcactga gtgacgaggt tagcaagtgg ttccggacag gcttgggggc cgaactggag 6960
gtggcactaa catctaggta tgaggtagag ggctgcaaaa gtatcctcat agccatggcc 7020
accttggcga gggacattaa ggcgtttaag aaattgagag gacctgttat acacctctac 7080
ggcggtccta gattggtgcg ttaatacaca gaattctgat tggatcatag cgcactatta 7140
taggatccag atcccgggta attaattgaa ttacatccct acgcaaacgt tttacggccg 7200
ccggtggcgc ccgcgcccgg cggcccgtcc ttggccgttg caggccactc cggtggctcc 7260
cgtcgtcccc gacttccagg cccagcagat gcagcaactc atcagcgccg taaatgcgct 7320
gacaatgaga cagaacgcaa ttgctcctgc taggcctccc aaaccaaaga agaagaagac 7380
aaccaaacca aagccgaaaa cgcagcccaa gaagatcaac ggaaaaacgc agcagcaaaa 7440
gaagaaagac aagcaagccg acaagaagaa gaagaaaccc ggaaaaagag aaagaatgtg 7500
catgaagatt gaaaatgact gtatcttcgt atgcggctag ccacagtaac gtagtgtttc 7560
cagacatgtc gggcaccgca ctatcatggg tgcagaaaat ctcgggtggt ctgggggcct 7620
tcgcaatcgg cgctatcctg gtgctggttg tggtcacttg cattgggctc cgcagataag 7680
ttagggtagg caatggcatt gatatagcaa gaaaattgaa aacagaaaaa gttagggtaa 7740
```

FIG.3F

```
gcaatggcat ataaccataa ctgtataact tgtaacaaag cgcaacaaga cctgcgcaat 7800
tggccccgtg gtccgcctca cggaaactcg gggcaactca tattgacaca ttaattggca 7860
ataattggaa gcttacataa gcttaattcg acgaataatt ggatttttat tttattttgc 7920
aattggtttt taatatttcc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 7980
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa                                  8010
```

FIG.4A

Nucleotide sequence of pSFVlink

```
gatggcggat gtgtgacata cacgacgcca aaagattttg ttccagctcc tgccacctcc 60
gctacgcgag agattaacca cccacgatgg ccgccaaagt gcatgttgat attgaggctg 120
acagcccatt catcaagtct ttgcagaagg catttccgtc gttcgaggtg gagtcattgc 180
aggtcacacc aaatgaccat gcaaatgcca gagcattttc gcacctggct accaaattga 240
tcgagcagga gactgacaaa gacacactca tcttggatat cggcagtgcg ccttccagga 300
gaatgatgtc tacgcacaaa taccactgcg tatgccctat gcgcagcgca gaagaccccg 360
aaaggctcga tagctacgca aagaaactgg cagcggcctc cgggaaggtg ctggatagag 420
agatcgcagg aaaaatcacc gacctgcaga ccgtcatggc tacgccagac gctgaatctc 480
ctacctttg cctgcataca gacgtcacgt gtcgtacggc agccgaagtg gccgtatacc 540
aggacgtgta tgctgtacat gcaccaacat cgctgtacca tcaggcgatg aaaggtgtca 600
gaacggcgta ttggattggg tttgacacca ccccgtttat gtttgacgcg ctagcaggcg 660
cgtatccaac ctacgccaca aactgggccg acgagcaggt gttacaggcc aggaacatag 720
gactgtgtgc agcatccttg actgagggaa gactcggcaa actgtccatt ctccgcaaga 780
agcaattgaa accttgcgac acagtcatgt tctcggtagg atctacattg tacactgaga 840
gcagaaagct actgaggagc tggcacttac cctccgtatt ccacctgaaa ggtaaacaat 900
cctttacctg taggtgcgat accatcgtat catgtgaagg gtacgtagtt aagaaaatca 960
ctatgtgccc cggcctgtac ggtaaaacgg tagggtacgc cgtgacgtat cacgcggagg 1020
gattcctagt gtgcaagacc acagacactg tcaaaggaga aagagtctca ttccctgtat 1080
gcacctacgt cccctcaacc atctgtgatc aaatgactgg catactagcg accgacgtca 1140
caccggagga cgcacagaag ttgttagtgg gattgaatca gaggatagtt gtgaacggaa 1200
gaacacagcg aaacactaac acgatgaaga actatctgct tccgattgtg gccgtcgcat 1260
ttagcaagtg ggcgagggaa tacaaggcag accttgatga tgaaaaacct ctgggtgtcc 1320
gagagaggtc acttacttgc tgctgcttgt gggcatttaa aacgaggaag atgcacacca 1380
tgtacaagaa accagacacc cagacaatag tgaaggtgcc ttcagagttt aactcgttcg 1440
```

FIG.4B

```
tcatcccgag cctatggtct acaggcctcg caatcccagt cagatcacgc attaagatgc 1500
ttttggccaa gaagaccaag cgagagttaa tacctgttct cgacgcgtcg tcagccaggg 1560
atgctgaaca agaggagaag gagaggttgg aggccgagct gactagagaa gccttaccac 1620
ccctcgtccc catcgcgccg gcggagacgg gagtcgtcga cgtcgacgtt gaagaactag 1680
agtatcacgc aggtgcaggg gtcgtggaaa cacctcgcag cgcgttgaaa gtcaccgcac 1740
agccgaacga cgtactacta ggaaattacg tagttctgtc cccgcagacc gtgctcaaga 1800
gctccaagtt ggcccccgtg caccctctag cagagcaggt gaaaataata acacataacg 1860
ggagggccgg cggttaccag gtcgacggat atgacggcag ggtcctacta ccatgtggat 1920
cggccattcc ggtccctgag tttcaagctt tgagcgagag cgccactatg gtgtacaacg 1980
aaagggagtt cgtcaacagg aaactatacc atattgccgt tcacggaccg tcgctgaaca 2040
ccgacgagga gaactacgag aaagtcagag ctgaaagaac tgacgccgag tacgtgttcg 2100
acgtagataa aaaatgctgc gtcaagagag aggaagcgtc gggtttggtg ttggtgggag 2160
agctaaccaa ccccccgttc catgaattcg cctacgaagg gctgaagatc aggccgtcgg 2220
caccatataa gactacagta gtaggagtct ttggggttcc gggatcaggc aagtctgcta 2280
ttattaagag cctcgtgacc aaacacgatc tggtcaccag cggcaagaag gagaactgcc 2340
aggaaatagt taacgacgtg aagaagcacc gcgggaaggg gacaagtagg gaaaacagtg 2400
actccatcct gctaaacggg tgtcgtcgtg ccgtggacat cctatatgtg gacgaggctt 2460
tcgcttgcca ttccggtact ctgctggccc taattgctct tgttaaacct cggagcaaag 2520
tggtgttatg cggagacccc aagcaatgcg gattcttcaa tatgatgcag cttaaggtga 2580
acttcaacca caacatctgc actgaagtat gtcataaaag tatatccaga cgttgcacgc 2640
gtccagtcac ggccatcgtg tctacgttgc actacggagg caagatgcgc acgaccaacc 2700
cgtgcaacaa acccataatc atagacacca caggacagac caagcccaag ccaggagaca 2760
tcgtgttaac atgcttccga ggctgggcaa agcagctgca gttggactac cgtggacacg 2820
aagtcatgac agcagcagca tctcagggcc tcacccgcaa aggggtatac gccgtaaggc 2880
agaaggtgaa tgaaaatccc ttgtatgccc ctgcgtcgga gcacgtgaat gtactgctga 2940
cgcgcactga ggataggctg gtgtggaaaa cgctggccgg cgatccctgg attaaggtcc 3000
```

FIG.4C

tatcaaacat tccacagggt aactttacgg ccacattgga agaatggcaa gaagaacacg 3060

acaaaataat gaaggtgatt gaaggaccgg ctgcgcctgt ggacgcgttc cagaacaaag 3120

cgaacgtgtg ttgggcgaaa agcctggtgc ctgtcctgga cactgccgga atcagattga 3180

cagcagagga gtggagcacc ataattacag catttaagga ggacagagct tactctccag 3240

tggtggcctt gaatgaaatt tgcaccaagt actatggagt tgacctggac agtggcctgt 3300

tttctgcccc gaaggtgtcc ctgtattacg agaacaacca ctgggataac agacctggtg 3360

gaaggatgta tggattcaat gccgcaacag ctgccaggct ggaagctaga catacc ttcc 3420

tgaaggggca gtggcatacg ggcaagcagg cagttatcgc agaaagaaaa atccaaccgc 3480

tttctgtgct ggacaatgta attcctatca accgcaggct gccgcacgcc ctggtggctg 3540

agtacaagac ggttaaaggc agtagggttg agtggctggt caataaagta agagggtacc 3600

acgtcctgct ggtgagtgag tacaacctgg ctttgcctcg acgcagggtc acttggttgt 3660

caccgctgaa tgtcacaggc gccgataggt gctacgacct aagtttagga ctgccggctg 3720

acgccggcag gttcgacttg gtctttgtga acattcacac ggaattcaga atccaccact 3780

accagcagtg tgtcgaccac gccatgaagc tgcagatgct tggggagat gcgctacgac 3840

tgctaaaacc cggcggcatc ttgatgagag cttacggata cgccgataaa atcagcgaag 3900

ccgttgtttc ctccttaagc agaaagttct cgtctgcaag agtgttgcgc ccggattgtg 3960

tcaccagcaa tacagaagtg ttcttgctgt tctccaactt tgacaacgga aagagaccct 4020

ctacgctaca ccagatgaat accaagctga gtgccgtgta tgccggagaa gccatgcaca 4080

cggccgggtg tgcaccatcc tacagagtta agagagcaga catagccacg tgcacagaag 4140

cggctgtggt taacgcagct aacgcccgtg gaactgtagg ggatggcgta tgcagggccg 4200

tggcgaagaa atggccgtca gcctttaagg gagcagcaac accagtgggc acaattaaaa 4260

cagtcatgtg cggctcgtac cccgtcatcc acgctgtagc gcctaatttc tctgccacga 4320

ctgaagcgga aggggaccgc gaattggccg ctgtctaccg ggcagtggcc gccgaagtaa 4380

acagactgtc actgagcagc gtagccatcc cgctgctgtc cacaggagtg ttcagcggcg 4440

gaagagatag gctgcagcaa tccctcaacc atctattcac agcaatggac gccacggacg 4500

ctgacgtgac catctactgc agagacaaaa gttgggagaa gaaaatccag gaagccattg 4560

FIG.4D

```
acatgaggac ggctgtggag ttgctcaatg atgacgtgga gctgaccaca gacttggtga 4620
gagtgcaccc ggacagcagc ctggtgggtc gtaagggcta cagtaccact gacgggtcgc 4680
tgtactcgta ctttgaaggt acgaaattca accaggctgc tattgatatg gcagagatac 4740
tgacgttgtg gcccagactg caagaggcaa acgaacagat atgcctatac gcgctgggcg 4800
aaacaatgga caacatcaga tccaaatgtc cggtgaacga ttccgattca tcaacacctc 4860
ccaggacagt gccctgcctg tgccgctacg caatgacagc agaacggatc gcccgcctta 4920
ggtcacacca agttaaaagc atggtggttt gctcatcttt tcccctcccg aaataccatg 4980
tagatggggt gcagaaggta aagtgcgaga aggttctcct gttcgacccg acggtacctt 5040
cagtggttag tccgcggaag tatgccgcat ctacgacgga ccactcagat cggtcgttac 5100
gagggtttga cttggactgg accaccgact cgtcttccac tgccagcgat accatgtcgc 5160
tacccagttt gcagtcgtgt gacatcgact cgatctacga gccaatggct cccatagtag 5220
tgacggctga cgtacaccct gaacccgcag gcatcgcgga cctggcggca gatgtgcacc 5280
ctgaacccgc agaccatgtg gacctcgaga acccgattcc tccaccgcgc ccgaagagag 5340
ctgcatacct tgcctcccgc gcggcggagc gaccggtgcc ggcgccgaga aagccgacgc 5400
ctgccccaag gactgcgttt aggaacaagc tgcctttgac gttcggcgac tttgacgagc 5460
acgaggtcga tgcgttggcc tccgggatta ctttcggaga cttcgacgac gtcctgcgac 5520
taggccgcgc gggtgcatat attttctcct cggacactgg cagcggacat ttacaacaaa 5580
aatccgttag gcagcacaat ctccagtgcg cacaactgga tgcggtccag gaggagaaaa 5640
tgtacccgcc aaaattggat actgagaggg agaagctgtt gctgctgaaa atgcagatgc 5700
acccatcgga ggctaataag agtcgatacc agtctcgcaa agtggagaac atgaaagcca 5760
cggtggtgga caggctcaca tcgggggcca gattgtacac gggagcggac gtaggccgca 5820
taccaacata cgcggttcgg taccccgcc ccgtgtactc ccctaccgtg atcgaaagat 5880
tctcaagccc cgatgtagca atcgcagcgt gcaacgaata cctatccaga aattacccaa 5940
cagtggcgtc gtaccagata acagatgaat acgacgcata cttggacatg gttgacgggt 6000
cggatagttg cttggacaga gcgacattct gcccggcgaa gctccggtgc tacccgaaac 6060
atcatgcgta ccaccagccg actgtacgca gtgccgtccc gtcacccttt cagaacacac 6120
```

FIG.4E

tacagaacgt	gctagcggcc	gccaccaaga	gaaactgcaa	cgtcacgcaa	atgcgagaac	6180
tacccaccat	ggactcggca	gtgttcaacg	tggagtgctt	caagcgctat	gcctgctccg	6240
gagaatattg	ggaagaatat	gctaaacaac	ctatccggat	aaccactgag	aacatcacta	6300
cctatgtgac	caaattgaaa	ggcccgaaag	ctgctgcctt	gttcgctaag	acccacaact	6360
tggttccgct	gcaggaggtt	cccatggaca	gattcacggt	cgacatgaaa	cgagatgtca	6420
aagtcactcc	agggacgaaa	cacacagagg	aaagacccaa	agtccaggta	attcaagcag	6480
cggagccatt	ggcgaccgct	tacctgtgcg	gcatccacag	ggaattagta	aggagactaa	6540
atgctgtgtt	acgccctaac	gtgcacacat	tgtttgatat	gtcggccgaa	gactttgacg	6600
cgatcatcgc	ctctcacttc	cacccaggag	acccggttct	agagacggac	attgcatcat	6660
tcgacaaaag	ccaggacgac	tccttggctc	ttacaggttt	aatgatcctc	gaagatctag	6720
gggtggatca	gtacctgctg	gacttgatcg	aggcagcctt	tggggaaata	tccagctgtc	6780
acctaccaac	tggcacgcgc	ttcaagttcg	gagctatgat	gaaatcgggc	atgtttctga	6840
ctttgtttat	taacactgtt	ttgaacatca	ccatagcaag	cagggtactg	gagcagagac	6900
tcactgactc	cgcctgtgcg	gccttcatcg	gcgacgacaa	catcgttcac	ggagtgatct	6960
ccgacaagct	gatggcggag	aggtgcgcgt	cgtgggtcaa	catggaggtg	aagatcattg	7020
acgctgtcat	gggcgaaaaa	cccccatatt	tttgtggggg	attcatagtt	tttgacagcg	7080
tcacacagac	cgcctgccgt	gtttcagacc	cacttaagcg	cctgttcaag	ttgggtaagc	7140
cgctaacagc	tgaagacaag	caggacgaag	acaggcgacg	agcactgagt	gacgaggtta	7200
gcaagtggtt	ccggacaggc	ttgggggccg	aactggaggt	ggcactaaca	tctaggtatg	7260
aggtagaggg	ctgcaaaagt	atcctcatag	ccatggccac	cttggcgagg	gacattaagg	7320
cgtttaagaa	attgagagga	cctgttatac	acctctacgg	cggtcctaga	ttggtgcgtt	7380
aatacacaga	attctgattg	gatcatagcg	cactattata	ggatccagat	cccgggtaat	7440
taattgaatt	acatccctac	gcaaacgttt	tacggccgcc	ggtggcgccc	gcgcccggcg	7500
gcccgtcctt	ggccgttgca	ggccactccg	gtggctcccg	tcgtccccga	cttccaggcc	7560
cagcagatgc	agcaactcat	cagcgccgta	aatgcgctga	caatgagaca	gaacgcaatt	7620
gctcctgcta	ggcctcccaa	accaaagaag	aagaagacaa	ccaaaccaaa	gccgaaaacg	7680

FIG.4F

```
cagcccaaga agatcaacgg aaaaacgcag cagcaaaaga agaaagacaa gcaagccgac 7740
aagaagaaga agaaacccgg aaaaagagaa agaatgtgca tgaagattga aaatgactgt 7800
atcttcgtat gcggctagcc acagtaacgt agtgtttcca gacatgtcgg gcaccgcact 7860
atcatgggtg cagaaaatct cgggtggtct gggggccttc gcaatcggcg ctatcctggt 7920
gctggttgtg gtcacttgca ttgggctccg cagataagtt agggtaggca atggcattga 7980
tatagcaaga aaattgaaaa cagaaaaagt tagggtaagc aatggcatat aaccataact 8040
gtataacttg taacaaagcg caacaagacc tgcgcaattg gccccgtggt ccgcctcacg 8100
gaaactcggg gcaactcata ttgacacatt aattggcaat aattggaagc ttacataagc 8160
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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 8280
aaaaaaaact agtctgcatt aatgaatcgg ccaacgcgcg gggagaggcg gtttgcgtat 8340
tgggcgctct tccgcttcct cgctcactga ctcgctgcgc tcggtcgttc ggctgcggcg 8400
agcggtatca gctcactcaa aggcggtaat acggttatcc acagaatcag gggataacgc 8460
aggaaagaac atgtgagcaa aaggccagca aaaggccagg aaccgtaaaa aggccgcgtt 8520
gctggcgttt ttccataggc tccgcccccc tgacgagcat cacaaaaatc gacgctcaag 8580
tcagaggtgg cgaaacccga caggactata aagataccag gcgtttcccc ctggaagctc 8640
cctcgtgcgc tctcctgttc cgaccctgcc gcttaccgga tacctgtccg cctttctccc 8700
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cgttcgctcc aagctgggct gtgtgcacga accccccgtt cagcccgacc gctgcgcctt 8820
atccggtaac tatcgtcttg agtccaaccc ggtaagacac gacttatcgc cactggcagc 8880
agccactggt aacaggatta gcagagcgag gtatgtaggc ggtgctacag agttcttgaa 8940
gtggtggcct aactacggct acactagaag gacagtattt ggtatctgcg ctctgctgaa 9000
gccagttacc ttcggaaaaa gagttggtag ctcttgatcc ggcaaacaaa ccaccgctgg 9060
tagcggtggt ttttttgttt gcaagcagca gattacgcgc agaaaaaaag gatctcaaga 9120
agatcctttg atcttttcta cggggtctga cgctcagtgg aacgaaaact cacgttaagg 9180
gattttggtc atgagattat caaaaaggat cttcacctag atccttttaa attaaaaatg 9240
```

FIG.4G

```
aagttttaaa tcaatctaaa gtatatatga gtaaacttgg tctgacagtt accaatgctt 9300
aatcagtgag gcacctatct cagcgatctg tctatttcgt tcatccatag ttgcctgact 9360
ccccgtcgtg tagataacta cgatacggga gggcttacca tctggcccca gtgctgcaat 9420
gataccgcga gacccacgct caccggctcc agatttatca gcaataaacc agccagccgg 9480
aagggccgag cgcagaagtg gtcctgcaac tttatccgcc tccatccagt ctattaattg 9540
ttgccgggaa gctagagtaa gtagttcgcc agttaatagt ttgcgcaacg ttgttgccat 9600
tgctacaggc atcgtggtgt cacgctcgtc gtttggtatg gcttcattca gctccggttc 9660
ccaacgatca aggcgagtta catgatcccc catgttgtgc aaaaaagcgg ttagctcctt 9720
cggtcctccg atcgttgtca gaagtaagtt ggccgcagtg ttatcactca tggttatggc 9780
agcactgcat aattctctta ctgtcatgcc atccgtaaga tgcttttctg tgactggtga 9840
gtactcaacc aagtcattct gagaatagtg tatgcggcga ccgagttgct cttgcccggc 9900
gtcaatacgg gataataccg cgccacatag cagaacttta aaagtgctca tcattggaaa 9960
acgttcttcg gggcgaaaac tctcaaggat cttaccgctg ttgagatcca gttcgatgta 10020
acccactcgt gcacccaact gatcttcagc atcttttact ttcaccagcg tttctgggtg 10080
agcaaaaaca ggaaggcaaa atgccgcaaa aaagggaata agggcgacac ggaaatgttg 10140
aatactcata ctcttccttt ttcaatatta ttgaagcatt tatcagggtt attgtctcat 10200
gagcggatac atatttgaat gtatttagaa aaataaacaa ataggggttc cgcgcacatt 10260
tccccgaaaa gtgccacctg acgtctaaga aaccattatt atcatgacat taacctataa 10320
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acaagcccgt cagggcgcgt cagcgggtgt tggcgggtgt cggggctggc ttaactatgc 10500
ggcatcagag cagattgtac tgagagtgca ccatatcgac gctctccctt atgcgactcc 10560
tgcattagga agcagcccag tactaggttg aggccgttga gcaccgccgc cgcaaggaat 10620
ggtgcatgca aggagatggc gcccaacagt cccccggcca cggggcctgc caccataccc 10680
acgccgaaac aagcgctcat gagcccgaag tggcgagccc gatcttcccc atcggtgatg 10740
tcggcgatat aggcgccagc aaccgcacct gtggcgccgg tgatgccggc cacgatgcgt 10800
```

FIG.4H

```
ccggcgtaga ggatctggct agcgatgacc ctgctgattg gttcgctgac catttccggg 10860
gtgcggaacg gcgttaccag aaactcagaa ggttcgtcca accaaaccga ctctgacggc 10920
agtttacgag agagatgata gggtctgctt cagtaagcca gatgctacac aattaggctt 10980
gtacatattg tcgttagaac gcggctacaa ttaatacata accttatgta tcatacacat 11040
acgatttagg tgacactata                                            11060
```

Construction of pSFVlink

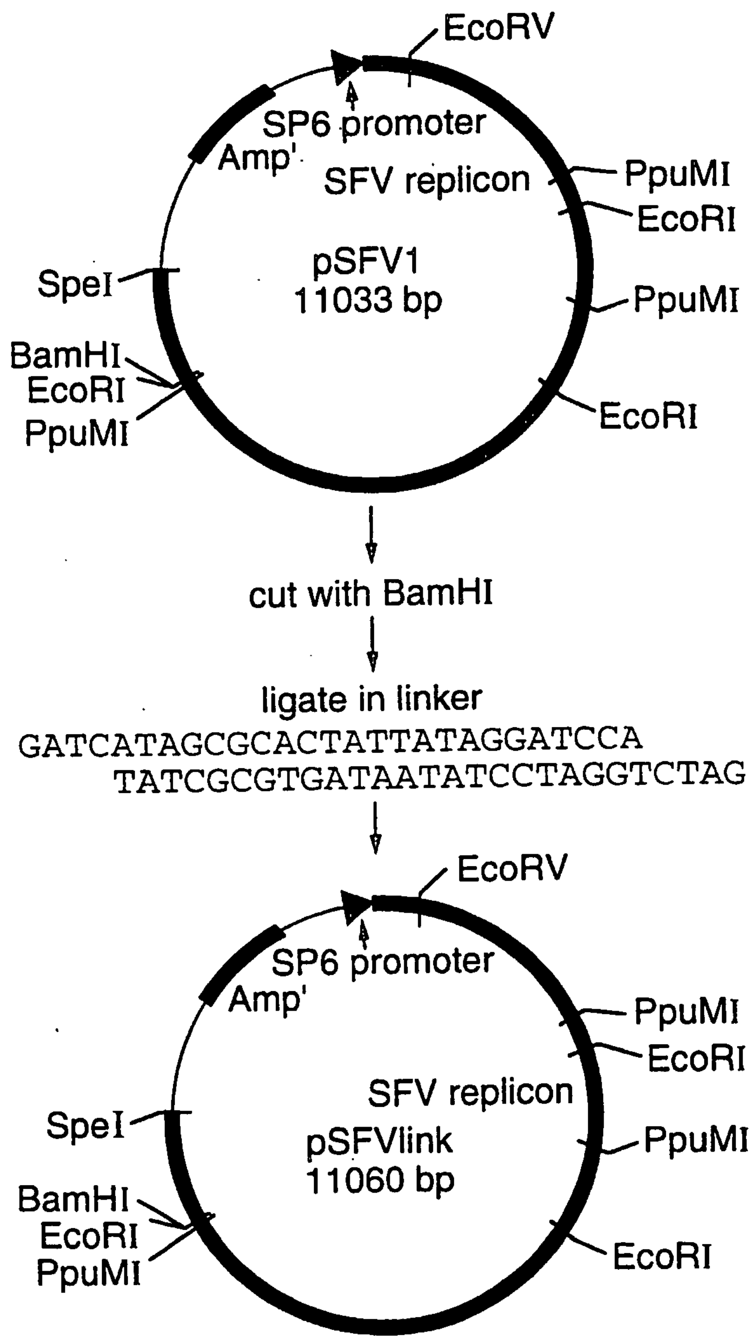

FIG.5

FIG.6A

Nucleotide Sequence of pMP76

```
attggctatt ggccattgca tacgttgtat ctatatcata atatgtacat ttatattggc 60
tcatgtccaa tatgaccgcc atgttgacat tgattattga ctagttatta atagtaatca 120
attacggggt cattagttca tagcccatat atggagttcc gcgttacata acttacggta 180
aatggcccgc ctcgtgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 240
ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggag tatttacggt 300
aaactgccca cttggcagta catcaagtgt atcatatgcc aagtccgccc cctattgacg 360
tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta cgggactttc 420
ctacttggca gtacatctac gtattagtca tcgctattac catggtgatg cggttttggc 480
agtacaccaa tgggcgtgga tagcggtttg actcacgggg atttccaagt ctccacccca 540
ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg ggactttcca aaatgtcgta 600
ataaccccgc cccgttgacg caaatgggcg gtaggcgtgt acggtgggag gtctatataa 660
gcagagctcg tttagtgaac cgtatggcgg atgtgtgaca tacacgacgc caaaagattt 720
tgttccagct cctgccacct ccgctacgcg agagattaac cacccacgat ggccgccaaa 780
gtgcatgttg atattgaggc tgacagccca ttcatcaagt ctttgcagaa ggcatttccg 840
tcgttcgagg tggagtcatt gcaggtcaca ccaaatgacc atgcaaatgc cagagcattt 900
tcgcacctgg ctaccaaatt gatcgagcag gagactgaca aagacacact catcttggat 960
atcggcagtg cgccttccag gagaatgatg tctacgcaca aataccactg cgtatgccct 1020
atgcgcagcg cagaagaccc cgaaaggctc gatagctacg caaagaaact ggcagcggcc 1080
tccgggaagg tgctggatag agagatcgca ggaaaaatca ccgacctgca gaccgtcatg 1140
gctacgccag acgctgaatc tcctaccttt tgcctgcata cagacgtcac gtgtcgtacg 1200
gcagccgaag tggccgtata ccaggacgtg tatgctgtac atgcaccaac atcgctgtac 1260
catcaggcga tgaaaggtgt cagaacggcg tattggattg ggtttgacac caccccgttt 1320
atgtttgacg cgctagcagg cgcgtatcca acctacgcca caaactgggc cgacgagcag 1380
```

FIG.6B

```
gtgttacagg ccaggaacat aggactgtgt gcagcatcct tgactgaggg aagactcggc 1440
aaactgtcca ttctccgcaa gaagcaattg aaaccttgcg acacagtcat gttctcggta 1500
ggatctacat tgtacactga gagcagaaag ctactgagga gctggcactt accctccgta 1560
ttccacctga aaggtaaaca atcctttacc tgtaggtgcg ataccatcgt atcatgtgaa 1620
gggtacgtag ttaagaaaat cactatgtgc cccggcctgt acggtaaaac ggtagggtac 1680
gccgtgacgt atcacgcgga gggattccta gtgtgcaaga ccacagacac tgtcaaagga 1740
gaaagagtct cattccctgt atgcacctac gtcccctcaa ccatctgtga tcaaatgact 1800
ggcatactag cgaccgacgt cacaccggag gacgcacaga agttgttagt gggattgaat 1860
cagaggatag ttgtgaacgg aagaacacag cgaaacacta acacgatgaa gaactatctg 1920
cttccgattg tggccgtcgc atttagcaag tgggcgaggg aatacaaggc agaccttgat 1980
gatgaaaaac ctctgggtgt ccgagagagg tcacttactt gctgctgctt gtgggcattt 2040
aaaacgagga agatgcacac catgtacaag aaaccagaca cccagacaat agtgaaggtg 2100
ccttcagagt ttaactcgtt cgtcatcccg agcctatggt ctacaggcct cgcaatccca 2160
gtcagatcac gcattaagat gcttttggcc aagaagacca agcgagagtt aatacctgtt 2220
ctcgacgcgt cgtcagccag ggatgctgaa caagaggaga aggagaggtt ggaggccgag 2280
ctgactagag aagccttacc acccctcgtc cccatcgcgc cggcggagac gggagtcgtc 2340
gacgtcgacg ttgaagaact agagtatcac gcaggtgcag gggtcgtgga aacacctcgc 2400
agcgcgttga aagtcaccgc acagccgaac gacgtactac taggaaatta cgtagttctg 2460
tccccgcaga ccgtgctcaa gagctccaag ttggcccccg tgcaccctct agcagagcag 2520
gtgaaaataa taacacataa cgggagggcc ggcggttacc aggtcgacgg atatgacggc 2580
agggtcctac taccatgtgg atcggccatt ccggtccctg agtttcaagc tttgagcgag 2640
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gttcacggac cgtcgctgaa caccgacgag gagaactacg agaaagtcag agctgaaaga 2760
actgacgccg agtacgtgtt cgacgtagat aaaaaatgct gcgtcaagag agaggaagcg 2820
tcgggtttgg tgttggtggg agagctaacc aaccccccgt tccatgaatt cgcctacgaa 2880
gggctgaaga tcaggccgtc ggcaccatat aagactacag tagtaggagt ctttggggtt 2940
```

FIG.6C

```
ccgggatcag gcaagtctgc tattattaag agcctcgtga ccaaacacga tctggtcacc 3000
agcggcaaga aggagaactg ccaggaaata gttaacgacg tgaagaagca ccgcgggaag 3060
gggacaagta gggaaaacag tgactccatc ctgctaaacg ggtgtcgtcg tgccgtggac 3120
atcctatatg tggacgaggc tttcgcttgc cattccggta ctctgctagc cctaattgct 3180
cttgttaaac ctcggagcaa agtggtgtta tgcggagacc ccaagcaatg cggattcttc 3240
aatatgatgc agcttaaggt gaacttcaac cacaacatct gcactgaagt atgtcataaa 3300
agtatatcca gacgttgcac gcgtccagtc acggccatcg tgtctacgtt gcactacgga 3360
ggcaagatgc gcacgaccaa cccgtgcaac aaacccataa tcatagacac cacaggacag 3420
accaagccca agccaggaga catcgtgtta acatgcttcc gaggctgggc aaagcagctg 3480
cagttggact accgtggaca cgaagtcatg acagcagcag catctcaggg cctcacccgc 3540
aaaggggtat acgccgtaag gcagaaggtg aatgaaaatc ccttgtatgc ccctgcgtcg 3600
gagcacgtga atgtactgct gacgcgcact gaggataggc tggtgtggaa aacgctggcc 3660
ggcgatccct ggattaaggt gagtttgggg acccttgatt gttctttctt tttcgctatt 3720
gtaaaattca tgttatatgg agggggcaaa gttttcaggg tgttgtttag aatgggaaga 3780
tgtcccttgt atcaccatgg accctcatga taattttgtt tctttcactt tctactctgt 3840
tgacaaccat tgtctcctct tattttcttt tcattttctg taactttttc gttaaacttt 3900
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ggctggcgtg gaaatattct tattggtaga aacaactaca tcctggtcat catcctgcct 4140
ttctctttat ggttacaatg atatacactg tttgagatga ggataaaata ctctgagtcc 4200
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ataatgaagg tgattgaagg accggctgcg cctgtggacg cgttccagaa caaagcgaac 4380
gtgtgttggg cgaaaagcct ggtgcctgtc ctggacactg ccggaatcag attgacagca 4440
gaggagtgga gcaccataat tacagcattt aaggaggaca gagcttactc tccagtggtg 4500
```

FIG.6D

```
gccttgaatg aaatttgcac caagtactat ggagttgacc tggacagtgg cctgttttct 4560
gccccgaagg tgtccctgta ttacgagaac aaccactggg ataacagacc tggtggaagg 4620
atgtatggat tcaatgccgc aacagctgcc aggctggaag ctagacatac cttcctgaag 4680
gggcagtggc atacgggcaa gcaggcagtt atcgcagaaa gaaaaatcca accgctttct 4740
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cagtgtgtcg accacgccat gaagctgcag atgcttgggg gagatgcgct acgactgcta 5100
aaacccggcg gcatcttgat gagagcttac ggatacgccg ataaaatcag cgaagccgtt 5160
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aagaaatggc cgtcagcctt taagggagca gcaacaccag tgggcacaat taaaacagtc 5520
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gcggaagggg accgcgaatt ggccgctgtc taccgggcag tggccgccga agtaaacaga 5640
ctgtcactga gcagcgtagc catcccgctg ctgtccacag gagtgttcag cggcggaaga 5700
gataggctgc agcaatccct caaccatcta ttcacagcaa tggacgccac ggacgctgac 5760
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tcgtactttg aaggtacgaa attcaaccag gctgctattg atatggcaga gatactgacg 6000
ttgtggccca gactgcaaga ggcaaacgaa cagatatgcc tatacgcgct gggcgaaaca 6060
```

FIG.6E

```
atggacaaca tcagatccaa atgtccggtg aacgattccg attcatcaac acctcccagg 6120
acagtgccct gcctgtgccg ctacgcaatg acagcagaac ggatcgcccg ccttaggtca 6180
caccaagtta aaagcatggt ggtttgctca tcttttcccc tcccgaaata ccatgtagat 6240
ggggtgcaga aggtaaagtg cgagaaggtt ctcctgttcg acccgacggt accttcagtg 6300
gttagtccgc ggaagtatgc cgcatctacg acggaccact cagatcggtc gttacgaggg 6360
tttgacttgg actggaccac cgactcgtct tccactgcca gcgataccat gtcgctaccc 6420
agtttgcagt cgtgtgacat cgactcgatc tacgagccaa tggctcccat agtagtgacg 6480
gctgacgtac accctgaacc cgcaggcatc gcggacctgg cggcagatgt gcaccctgaa 6540
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aacgtgctag cggccgccac caagagaaac tgcaacgtca cgcaaatgcg agaactaccc 7440
accatggact cggcagtgtt caacgtggag tgcttcaagc gctatgcctg ctccggagaa 7500
tattgggaag aatatgctaa acaacctatc cggataacca ctgagaacat cactacctat 7560
gtgaccaaat tgaaaggccc gaaagctgct gccttgttcg ctaagaccca caacttggtt 7620
```

FIG.6F

```
ccgctgcagg aggttcccat ggacagattc acggtcgaca tgaaacgaga tgtcaaagtc 7680
actccaggga cgaaacacac agaggaaaga cccaaagtcc aggtaattca agcagcggag 7740
ccattggcga ccgcttacct gtgcggcatc cacagggaat tagtaaggag actaaatgct 7800
gtgttacgcc ctaacgtgca cacattgttt gatatgtcgg ccgaagactt tgacgcgatc 7860
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gatcagtacc tgctggactt gatcgaggca gcctttgggg aaatatccag ctgtcaccta 8040
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cagaccgcct gccgtgtttc agacccactt aagcgcctgt tcaagttggg taagccgcta 8400
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tggttccgga caggcttggg ggccgaactg gaggtggcac taacatctag gtatgaggta 8520
gagggctgca aaagtatcct catagccatg gccaccttgg cgagggacat taaggcgttt 8580
aagaaattga gaggacctgt tatacacctc tacggcggtc ctagattggt gcgttaatac 8640
acagaattct gattggatca tagcgcacta ttataggatc cagatcccgg gtaattaatt 8700
gaattacatc cctacgcaaa cgttttacgg ccgccggtgg cgcccgcgcc cggcggcccg 8760
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gatgcagcaa ctcatcagcg ccgtaaatgc gctgacaatg agacagaacg caattgctcc 8880
tgctaggcct cccaaaccaa agaagaagaa gacaaccaaa ccaaagccga aaacgcagcc 8940
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gaagaagaaa cccggaaaaa gagaaagaat gtgcatgaag attgaaaatg actgtatctt 9060
cgtatgcggc tagccacagt aacgtagtgt ttccagacat gtcgggcacc gcactatcat 9120
gggtgcagaa aatctcgggt ggtctggggg ccttcgcaat cggcgctatc ctggtgctgg 9180
```

FIG.6G

```
ttgtggtcac ttgcattggg ctccgcagat aagttagggt aggcaatggc attgatatag 9240
caagaaaatt gaaaacagaa aaagttaggg taagcaatgg catataacca taactgtata 9300
acttgtaaca aagcgcaaca agacctgcgc aattggcccc gtggtccgcc tcacggaaac 9360
tcggggcaac tcatattgac acattaattg gcaataattg gaagcttaca taagcttaat 9420
tcgacgaata attggatttt tattttattt tgcaattggt tttaatattt tccaaaaaaa 9480
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 9540
aaacgggtcg gcatggcatc tccacctcct cgcggtccga cctgggcatc cgaaggagga 9600
cgcacgtcca ctcggatggc taagggagat cctgaactta acgctcgagt gccagccatc 9660
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ttcctaataa aatgaggaaa ttgcatcgca ttgtctgagt aggtgtcatt ctattctggg 9780
gggtggggtg gggcaggaca gcaaggggga ggattgggaa gacaatagca ggcatgctgg 9840
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gtgagggtta atttcgagct tggcgtaatc atggtcatag ctgtttcctg tgtgaaattg 10020
ttatccgctc acaattccac acaacatacg agccggaagc ataaagtgta aagcctgggg 10080
tgcctaatga gtgagctaac tcacattaat tgcgttgcgc tcactgcccg ctttccagtc 10140
gggaaacctg tcgtgccagc tgcattaatg aatcggccaa cgcgcgggga gaggcggttt 10200
gcgtattggg cgctcttccg cttcctcgct cactgactcg ctgcgctcgg tcgttcggct 10260
gcggcgagcg gtatcagctc actcaaaggc ggtaatacgg ttatccacag aatcagggga 10320
taacgcagga aagaacatgt gagcaaaagg ccagcaaaag gccaggaacc gtaaaaaggc 10380
cgcgttgctg gcgtttttcc ataggctccg cccccctgac gagcatcaca aaaatcgacg 10440
ctcaagtcag aggtggcgaa acccgacagg actataaaga taccaggcgt ttccccctgg 10500
aagctccctc gtgcgctctc ctgttccgac cctgccgctt accggatacc tgtccgcctt 10560
tctcccttcg ggaagcgtgg cgctttctca tagctcacgc tgtaggtatc tcagttcggt 10620
gtaggtcgtt cgctccaagc tgggctgtgt gcacgaaccc cccgttcagc ccgaccgctg 10680
cgccttatcc ggtaactatc gtcttgagtc caacccggta agacacgact tatcgccact 10740
```

FIG.6H

```
ggcagcagcc actggtaaca ggattagcag agcgaggtat gtaggcggtg ctacagagtt 10800
cttgaagtgg tggcctaact acggctacac tagaaggaca gtatttggta tctgcgctct 10860
gctgaagcca gttaccttcg gaaaaagagt tggtagctct tgatccggca aacaaaccac 10920
cgctggtagc ggtggttttt ttgtttgcaa gcagcagatt acgcgcagaa aaaaaggatc 10980
tcaagaagat cctttgatct tttctacggg gtctgacgct cagtggaacg aaaactcacg 11040
ttaagggatt ttggtcatga gattatcaaa aaggatcttc acctagatcc ttttaaatta 11100
aaaatgaagt tttaaatcaa tctaaagtat atatgagtaa acttggtctg acagttacca 11160
atgcttaatc agtgaggcac ctatctcagc gatctgtcta tttcgttcat ccatagttgc 11220
ctgactccgg gggggggggg cgctgaggtc tgcctcgtga agaaggtgtt gctgactcat 11280
accaggcctg aatcgcccca tcatccagcc agaaagtgag ggagccacgg ttgatgagag 11340
ctttgttgta ggtggaccag ttggtgattt tgaacttttg ctttgccacg gaacggtctg 11400
cgttgtcggg aagatgcgtg atctgatcct tcaactcagc aaaagttcga tttattcaac 11460
aaagccgccg tcccgtcaag tcagcgtaat gctctgccag tgttacaacc aattaaccaa 11520
ttctgattag aaaaactcat cgagcatcaa atgaaactgc aatttattca tatcaggatt 11580
atcaatacca tatttttgaa aaagccgttt ctgtaatgaa ggagaaaact caccgaggca 11640
gttccatagg atggcaagat cctggtatcg gtctgcgatt ccgactcgtc caacatcaat 11700
acaacctatt aatttcccct cgtcaaaaat aaggttatca agtgagaaat caccatgagt 11760
gacgactgaa tccggtgaga atggcaaaag cttatgcatt tctttccaga cttgttcaac 11820
aggccagcca ttacgctcgt catcaaaatc actcgcatca accaaaccgt tattcattcg 11880
tgattgcgcc tgagcgagac gaaatacgcg atcgctgtta aaaggacaat tacaaacagg 11940
aatcgaatgc aaccggcgca ggaacactgc cagcgcatca acaatatttt cacctgaatc 12000
aggatattct tctaatacct ggaatgctgt tttcccgggg atcgcagtgg tgagtaacca 12060
tgcatcatca ggagtacgga taaaatgctt gatggtcgga agaggcataa attccgtcag 12120
ccagtttagt ctgaccatct catctgtaac atcattggca acgctacctt tgccatgttt 12180
cagaaacaac tctggcgcat cgggcttccc atacaatcga tagattgtcg cacctgattg 12240
cccgacatta tcgcgagccc atttataccc atataaatca gcatccatgt tggaatttaa 12300
```

FIG.6I

```
tcgcggcctc gagcaagacg tttcccgttg aatatggctc ataacacccc ttgtattact 12360
gtttatgtaa gcagacagtt ttattgttca tgatgatata tttttatctt gtgcaatgta 12420
acatcagaga ttttgagaca caacgtggct ttcccccccc cccccgagct tgat       12474
```

CMV promoter 1 - 682
SFV replicon (before intron) 684 - 3678
Rabbit (-globin intron II 3679 - 4251
SFV replicon (after intron) 4252 - 9543
Hepatitis Delta virus ribozyme (antigenomic) 9544 - 9628
Kanamycin Gene 12342 - 11503
BamHI site for insertion of heterologous inserts 8677

Subcloning of the SFV replicon

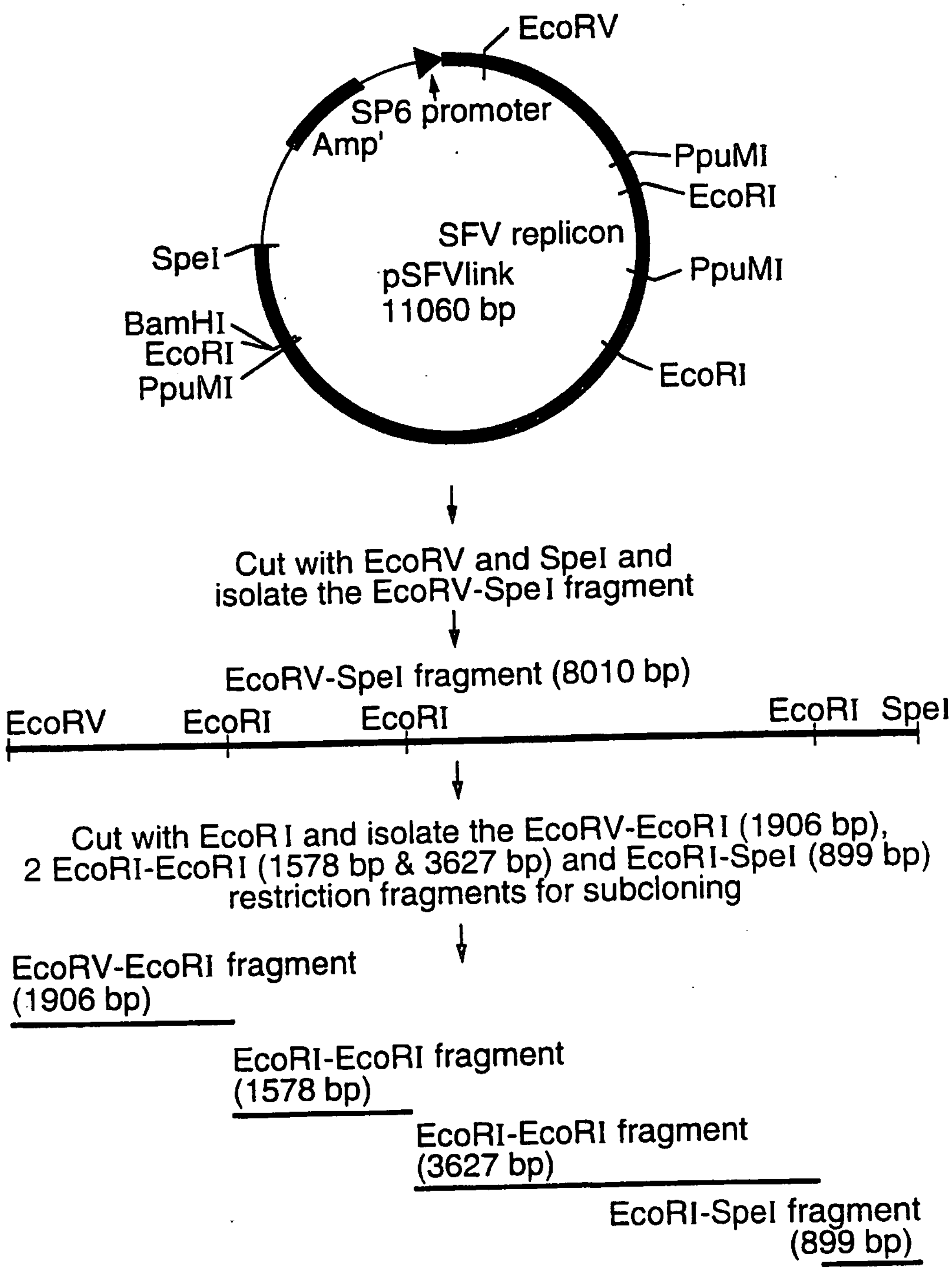


FIG.7

Construction of pMP76

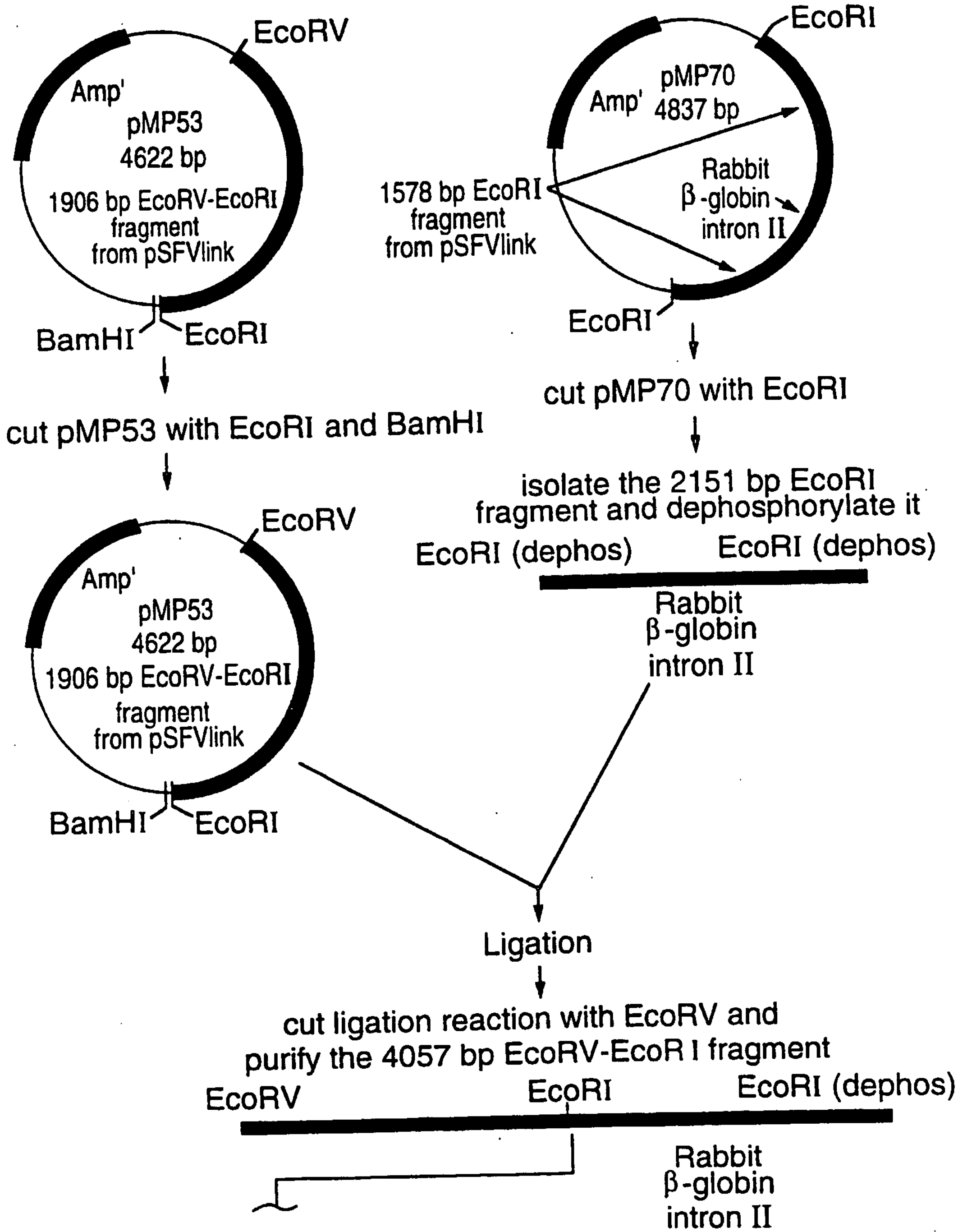


FIG.8A

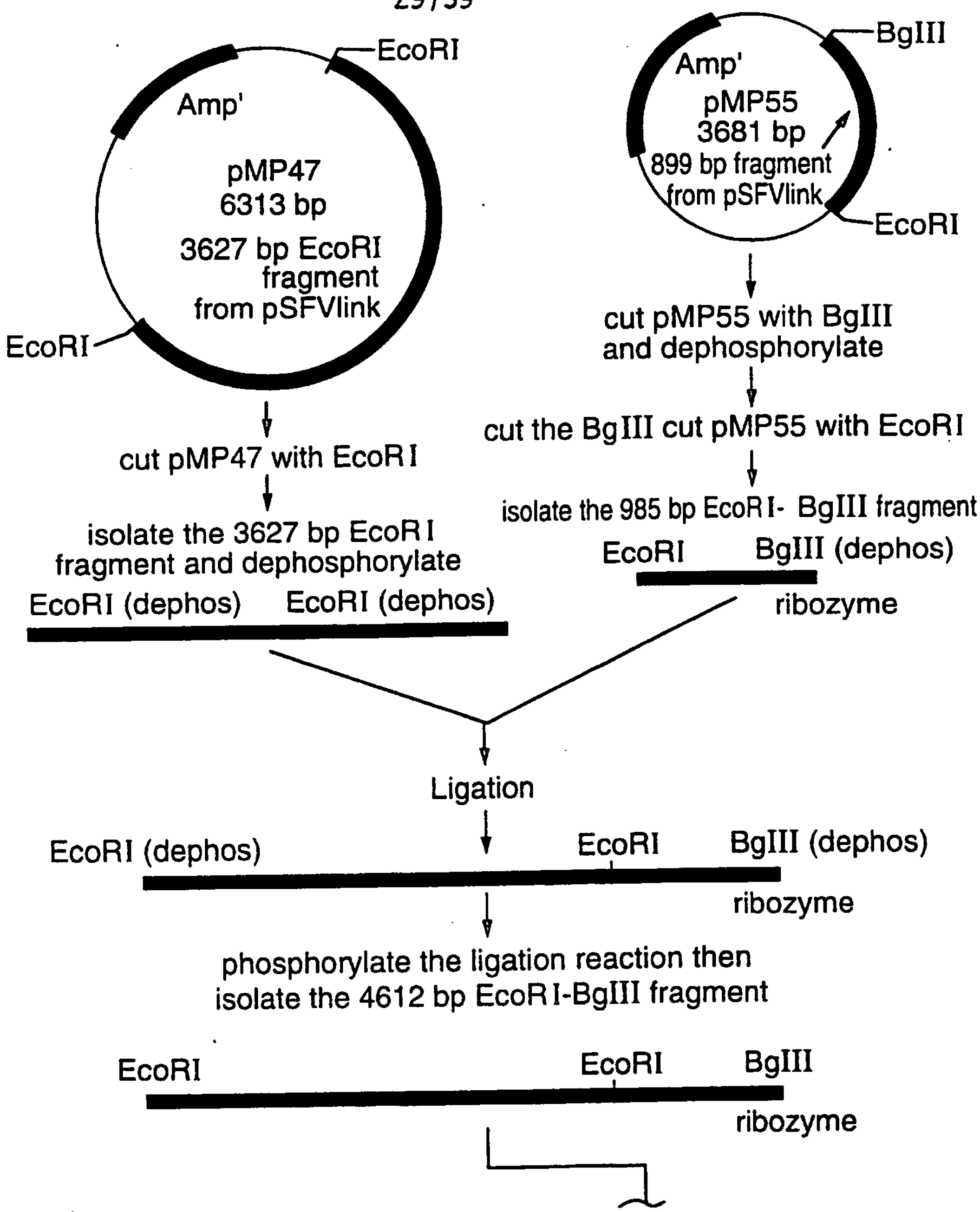

EcoRI
Amp'
pMP47
6313 bp
3627 bp EcoRI
fragment
from pSFVlink
EcoRI
BgIII
Amp'
pMP55
3681 bp
899 bp fragment
from pSFVlink
EcoRI
cut pMP55 with BgIII
and dephosphorylate
cut the BgIII cut pMP55 with EcoRI
isolate the 985 bp EcoRI- BgIII fragment
EcoRI
BgIII (dephos)
ribozyme
cut pMP47 with EcoRI
isolate the 3627 bp EcoRI
fragment and dephosphorylate
EcoRI (dephos)
EcoRI (dephos)
Ligation
EcoRI (dephos)
EcoRI
BgIII (dephos)
ribozyme
phosphorylate the ligation reaction then
isolate the 4612 bp EcoRI-BgIII fragment
EcoRI
EcoRI
BgIII
ribozyme

FIG.8B

Construction of pMP76

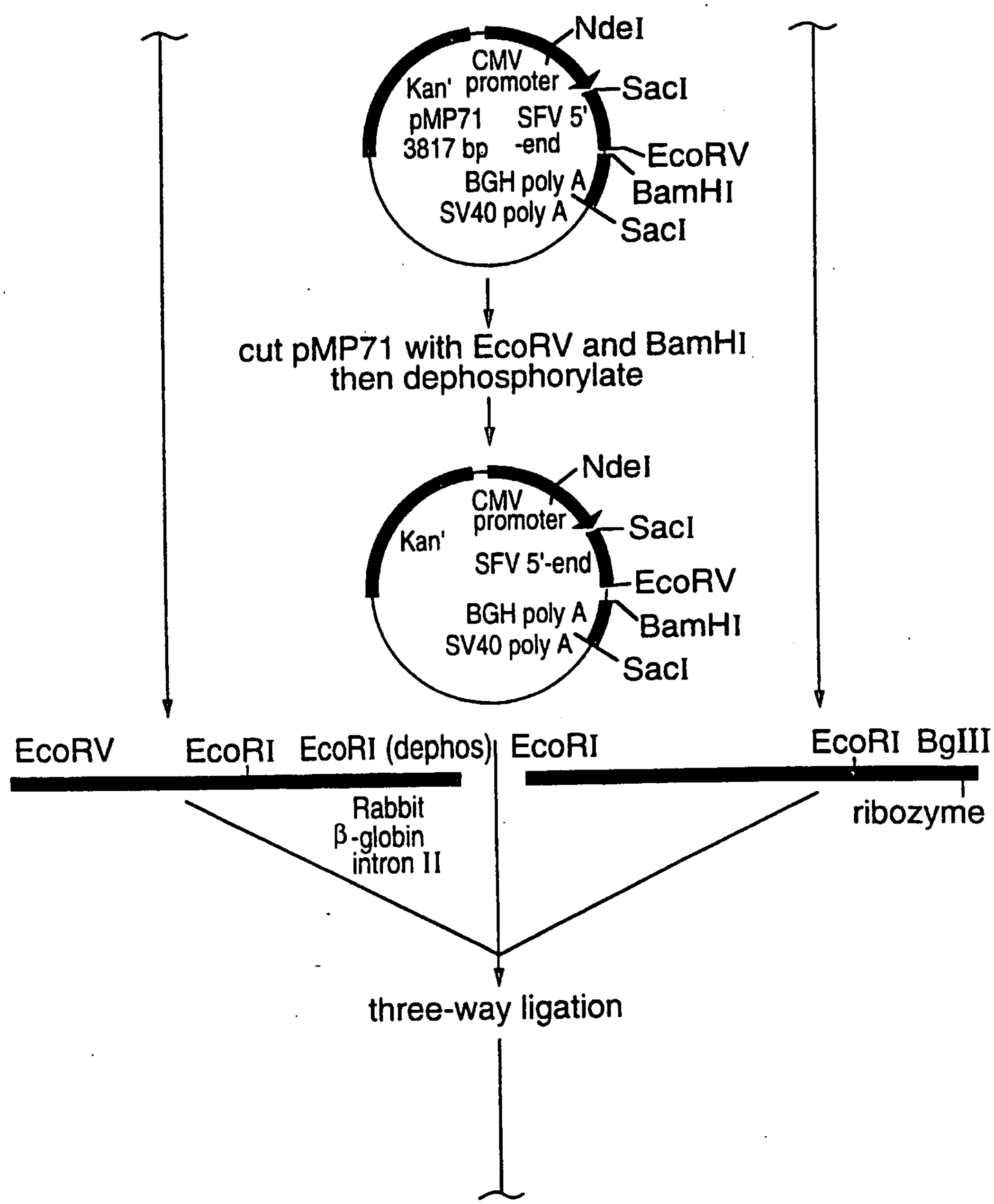


FIG.8C

Construction of pMP76 (cont'd)

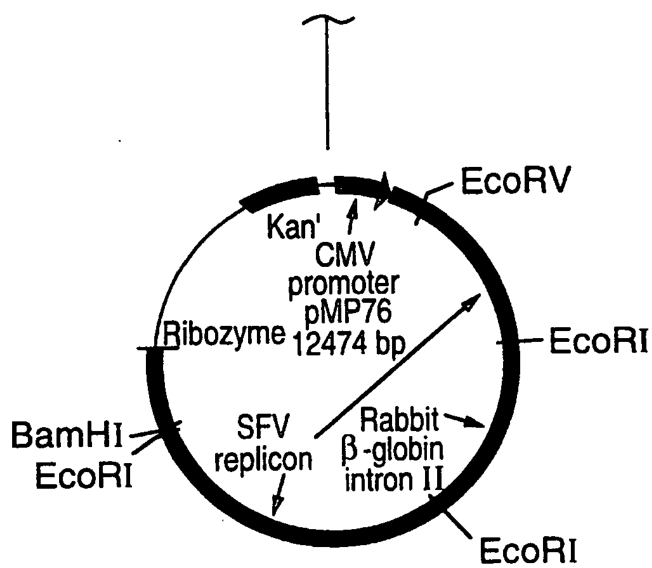


FIG.8D

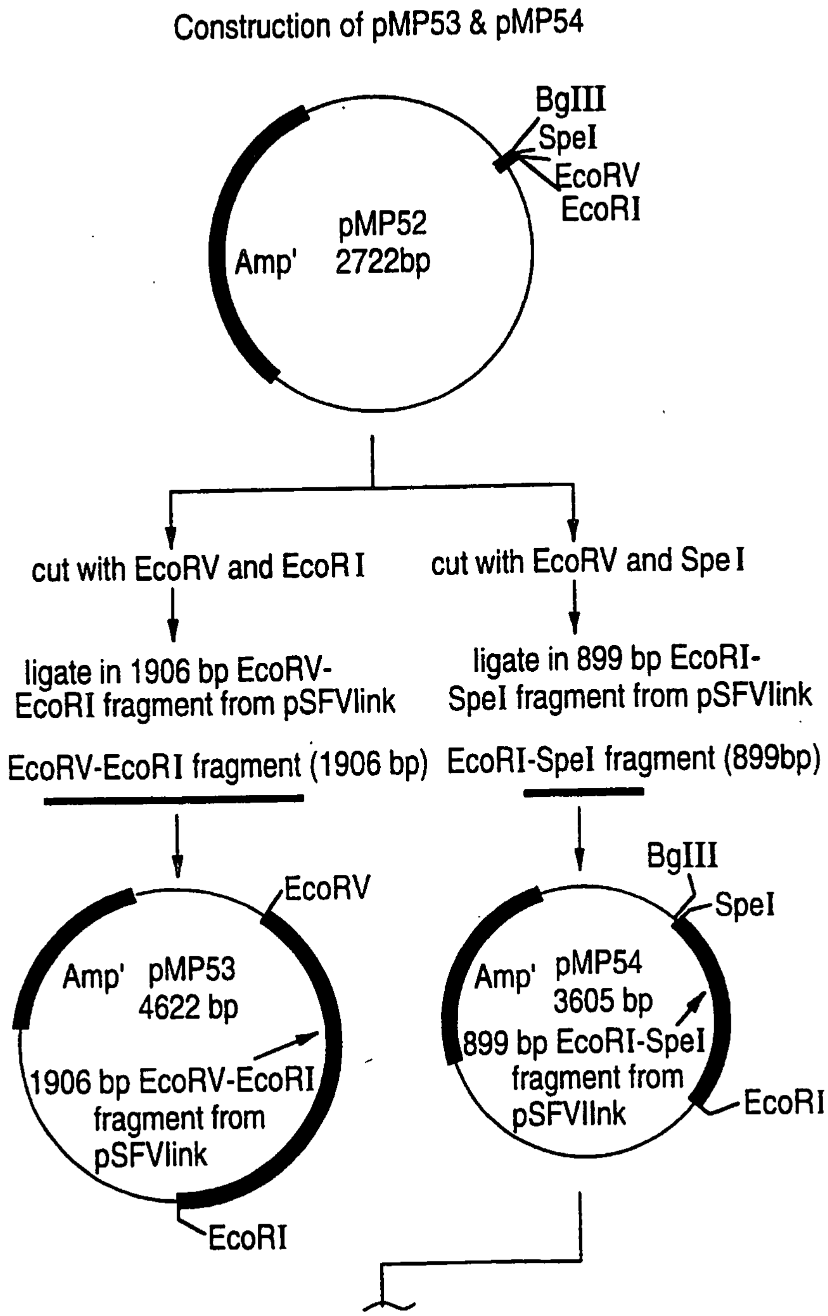

Construction of pMP53 & pMP54
BgIII
SpeI
EcoRV
EcoRI
pMP52
Amp'
2722bp
cut with EcoRV and EcoR I
cut with EcoRV and Spe I
ligate in 1906 bp EcoRV-
EcoRI fragment from pSFVlink
ligate in 899 bp EcoRI-
SpeI fragment from pSFVlink
EcoRV-EcoRI fragment (1906 bp)
EcoRI-SpeI fragment (899bp)
EcoRV
Amp'
pMP53
4622 bp
1906 bp EcoRV-EcoRI
fragment from
pSFVlink
EcoRI
BgIII
SpeI
Amp'
pMP54
3605 bp
899 bp EcoRI-SpeI
fragment from
pSFVIlnk
EcoRI

FIG.9A

Construction of pMP55

cut pMP54 with Spe I and make blunt-ended with Mung Bean nuclease

cut with BglII and dephosphorylate

ligate in phosphorylated linker-Hepatitis Delta virus ribozyme (antigenomic)

```
CGGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCA...
GCCCAGCCGTACCGTAGAGGTGGAGGAGCGCCAGGCTGGACCCGT...
        ...TCCGAAGGAGGACGCACGTCCACTCGGATGGCTAAGGGAGA
        ...AGGCTTCCTCCTGCGTGCAGGTGAGCCTACCGATTCCCTCTCTAG
```

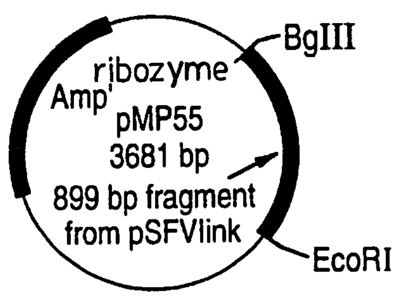
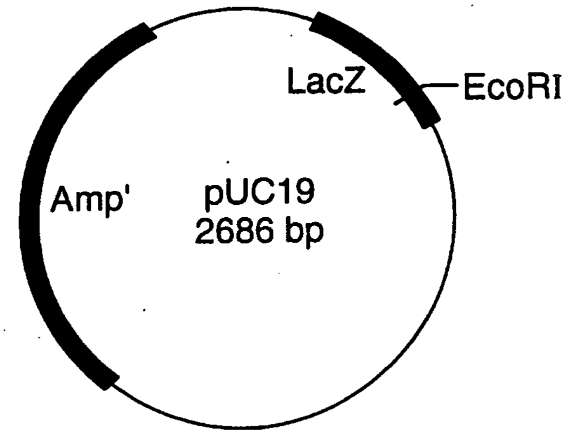


FIG.9B

Construction of pMP52



cut with EcoRI and dephosphorylate

ligate in phosphorylated linker

```
AATTCATGATATCATACTAGTATATAGATCT
          GTACTATAGTATGATCATATATCTAGATTAA
```

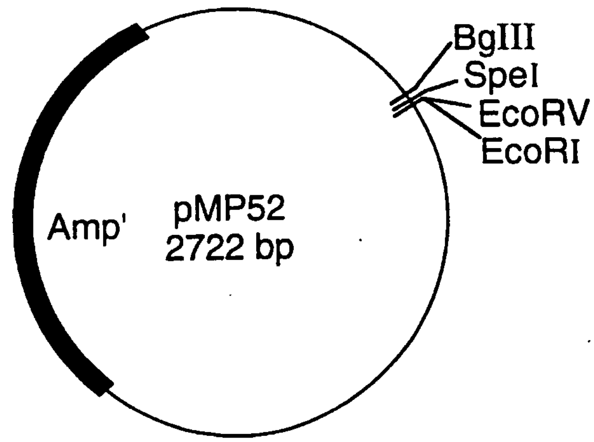


FIG.10

Construction of pMP46 & pMP47

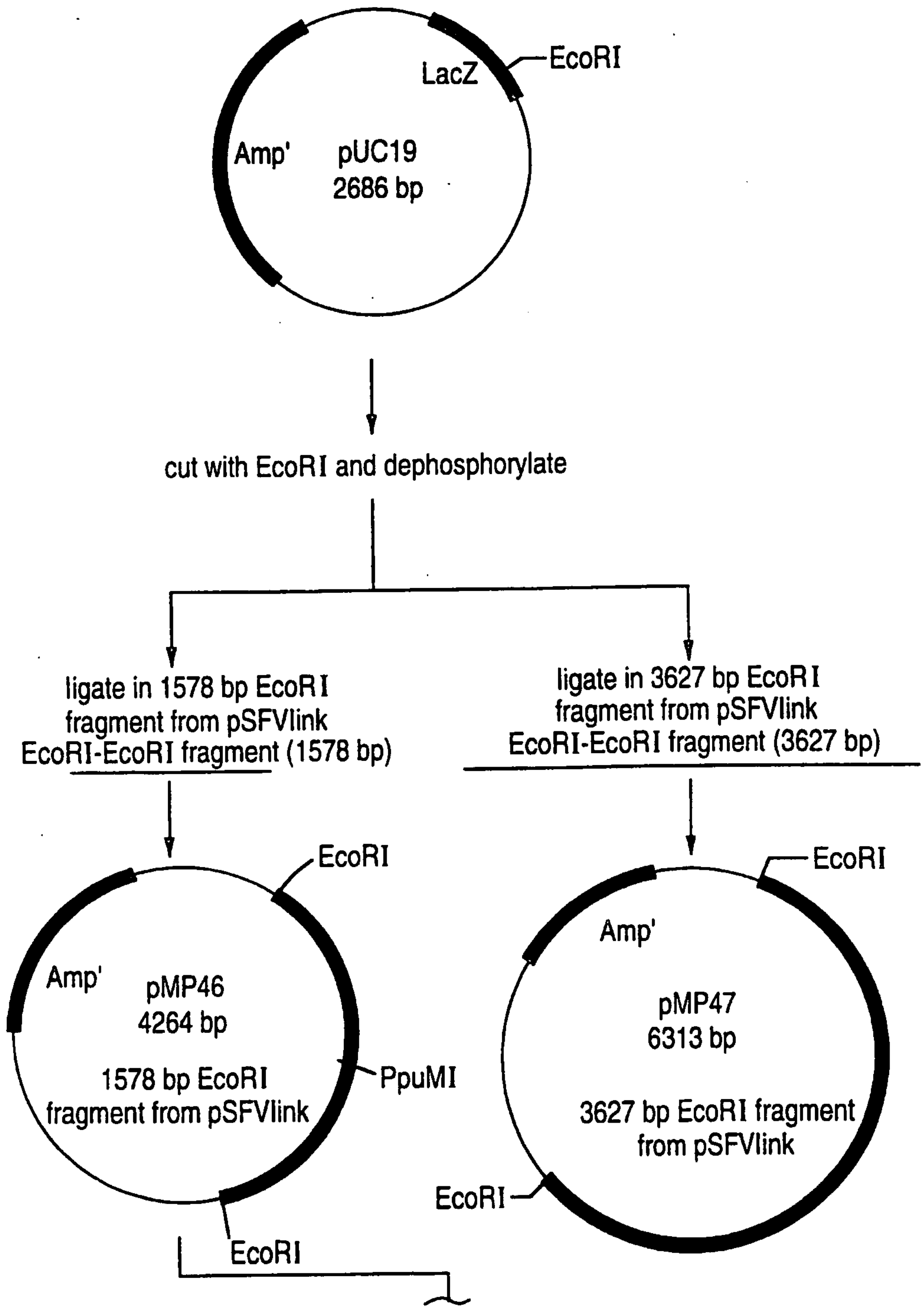


FIG.11A

Construction of pMP70

cut pMP46 with PpuMI

↓

Make blunt-ended with Mung Bean nuclease

↓

ligate with the rabbit β-globin Intron II PCR fragment In Fig.1 that was made blunt-ended with Mung Bean nuclease and was then phosphorylated

↓

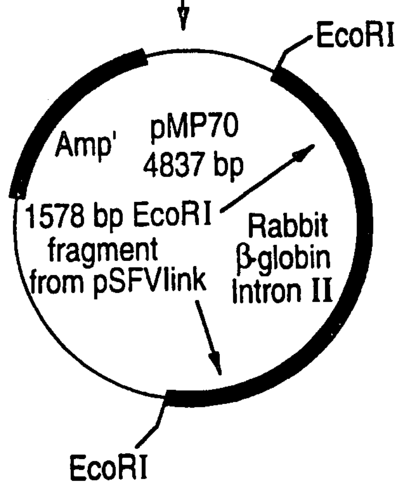


FIG.11B

Construction of pMP71

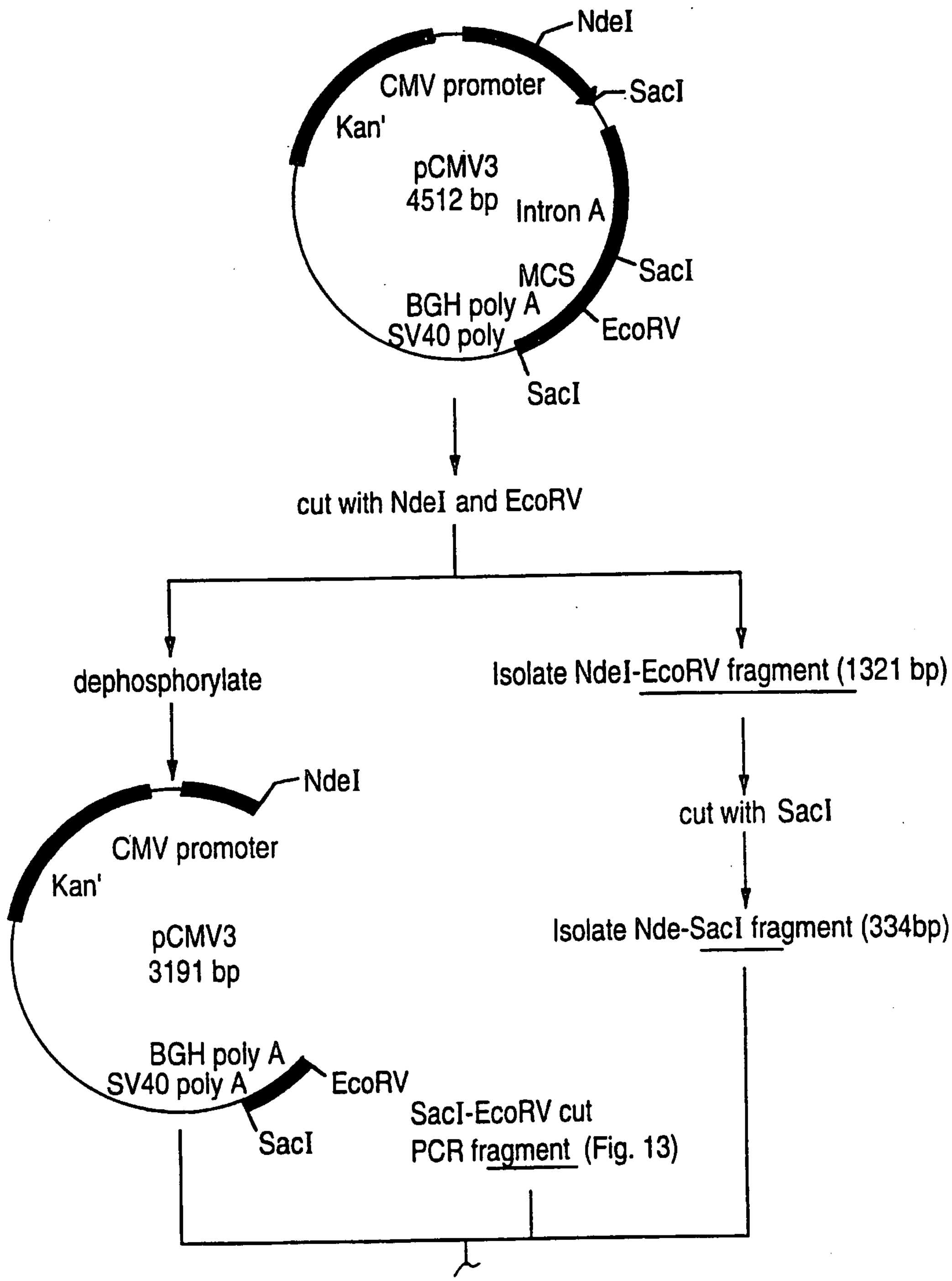


FIG.12A

Construction of pMP71 (cont'd)

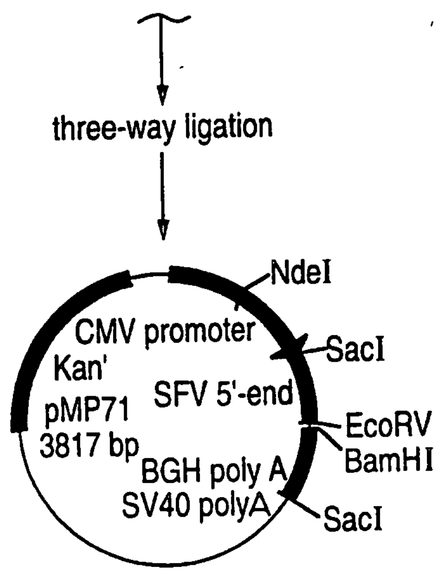


FIG.12B

FIG.13

```
1    CGTTTAGTGA ACCGTATGGC GGATGTGTGA CATACACGAC GCCAAAAGAT 50
51   TTTGTTCCAG CTCCTGCCAC CTCCGCTACG CGAGAGATTA ACCACCCACG 100
101  ATGGCCGCCA AAGTGCATGT TGATATTGAG GCTGACAGCC CATTCATCAA 150
151  GTCTTTGCAG AAGGCATTTC CGTCGTTCGA GGTGGAGTCA TTGCAGGTCA 200
201  CACCAAATGA CCATGCAAAT GCCAGAGCAT TTTCGCACCT GGCTACCAAA 250
251  TTGATCGAGC AGGAGACTGA CAAAGACACA CTCATCTTGG AT         292
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/01065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 27044 A (BIOPTION AB ;LILJESTROEM PETER (SE); GAROFF HENRIK (SE)) 12 October 1995 cited in the application see the whole document, especially page 8, lines 12-22	1-14
Y	WO 96 40945 A (CONNAUGHT LAB ;LI XIAOMAO (CA); EWASYSHYN MARY E (CA); SAMBHARA SU) 19 December 1996 cited in the application see the whole document, especially page 6, lines 2-9; page 14, lines 15-21; and page 23, lines 18-23	1-14
A	WO 96 17072 A (VIAGENE INC) 6 June 1996 see the whole document	1-14
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 April 1999	03/05/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Intern ›nal Application No
PCT/CA 98/01065

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine" VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document ---	1-14
A	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMLIKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document -----	1-14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/01065

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
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			AU	2155795	A	23-10-1995
			CA	2184261	A	12-10-1995
			EP	0753053	A	15-01-1997
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			AU	6117696	A	30-12-1996
			CA	2223610	A	19-12-1996
			EP	0832253	A	01-04-1998
			US	5843913	A	01-12-1998
			US	5880104	A	09-03-1999
WO 9617072	A	06-06-1996	AU	4594996	A	19-06-1996
			EP	0797679	A	01-10-1997
			US	5814482	A	29-09-1998
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			US	5789245	A	04-08-1998